

PHTHALAZINONE DERIVATIVES

The present invention relates to phthalazinone derivatives, and their use as pharmaceuticals. In particular, the present invention relates to the use of these compounds to inhibit the activity of the enzyme poly (ADP-ribose)polymerase, also known as poly(ADP-ribose)synthase and poly ADP-ribosyltransferase, and commonly referred to as PARP.

The mammalian enzyme PARP (a 113-kDa multidomain protein) has been implicated in the signalling of DNA damage through its ability to recognize and rapidly bind to DNA single or double strand breaks (D'Amours, *et al.*, *Biochem. J.*, **342**, 249-268 (1999)).

Several observations have led to the conclusion that PARP participates in a variety of DNA-related functions including gene amplification, cell division, differentiation, apoptosis, DNA base excision repair and also effects on telomere length and chromosome stability (d'Adda di Fagagna, *et al.*, *Nature Gen.*, **23(1)**, 76-80 (1999)).

Studies on the mechanism by which PARP modulates DNA repair and other processes has identified its importance in the formation of poly (ADP-ribose) chains within the cellular nucleus (Althaus, F.R. and Richter, C., *ADP-Ribosylation of Proteins: Enzymology and Biological Significance*, Springer-Verlag, Berlin (1987)). The DNA-bound, activated PARP utilizes NAD to synthesize poly (ADP-ribose) on a variety of nuclear target proteins, including topoisomerase, histones and PARP itself (Rhun, *et al.*, *Biochem. Biophys. Res. Commun.*, **245**, 1-10 (1998)).

Poly (ADP-ribosyl)ation has also been associated with malignant transformation. For example, PARP activity is higher in the isolated nuclei of SV40-transformed fibroblasts, while both leukemic cells and colon cancer cells show higher enzyme activity than the equivalent normal leukocytes and colon mucosa (Miwa, *et al.*, *Arch. Biochem. Biophys.*, **181**, 313-321 (1977); Burzio, *et al.*, *Proc. Soc. Exp. Biol. Med.*, **149**, 933-938 (1975); and Hirai, *et al.*, *Cancer Res.*, **43**, 3441-3446 (1983)).

A number of low-molecular-weight inhibitors of PARP have been used to elucidate the functional role of poly (ADP-ribosyl)ation in DNA repair. In cells treated with alkylating agents, the inhibition of PARP leads to a marked increase in DNA-strand breakage and cell killing (Durkacz, *et al.*, *Nature*, **283**, 593-596 (1980); Berger, N.A., *Radiation Research*, **101**, 4-14 (1985)).

Subsequently, such inhibitors have been shown to enhance the effects of radiation response by suppressing the repair of potentially lethal damage (Ben-Hur, *et al.*, *British Journal of Cancer*, **49** (Suppl. VI), 34-42 (1984); Schlicker, *et al.*, *Int. J. Radiat. Biol.*, **75**, 91-100 (1999)). PARP inhibitors have been reported to be effective in radio sensitising hypoxic tumour cells (US 5,032,617; US 5,215,738 and US 5,041,653).

Furthermore, PARP knockout (PARP -/-) animals exhibit genomic instability in response to alkylating agents and γ -irradiation (Wang, *et al.*, *Genes Dev.*, **9**, 509-520 (1995); Menissier de Murcia, *et al.*, *Proc. Natl. Acad. Sci. USA*, **94**, 7303-7307 (1997)).

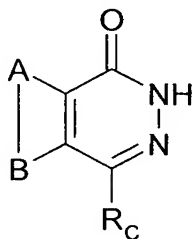
A role for PARP has also been demonstrated in certain vascular diseases, septic shock, ischaemic injury and neurotoxicity (Cantoni, *et al.*, *Biochim. Biophys. Acta*, **1014**, 1-7 (1989); Szabo, *et al.*, *J. Clin. Invest.*, **100**, 723-735 (1997)). Oxygen radical DNA damage that leads to strand breaks in DNA, which are subsequently recognised by PARP, is a major contributing factor to such disease states as shown by PARP inhibitor studies (Cosi, *et al.*, *J. Neurosci. Res.*, **39**, 38-46 (1994); Said, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 4688-4692 (1996)). More recently, PARP has been demonstrated to play a role in the pathogenesis of haemorrhagic shock (Liaudet, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, **97**(3), 10203-10208 (2000)).

It has also been demonstrated that efficient retroviral infection of mammalian cells is blocked by the inhibition of PARP activity. Such inhibition of recombinant retroviral vector infections was shown to occur in various different cell types (Gaken, *et al.*, *J. Virology*, **70**(6), 3992-4000 (1996)). Inhibitors of PARP have thus been developed

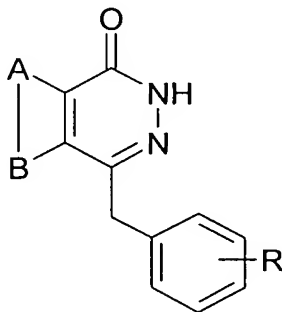
for the use in anti-viral therapies and in cancer treatment (WO 91/18591).

Moreover, PARP inhibition has been speculated to delay the onset of aging characteristics in human fibroblasts (Rattan and Clark, *Biochem. Biophys. Res. Comm.*, **201(2)**, 665-672 (1994)). This may be related to the role that PARP plays in controlling telomere function (d'Adda di Fagagna, *et al.*, *Nature Gen.*, **23(1)**, 76-80 (1999)).

- Some of the present inventors have previously described (WO 02/36576) a class of 1(2H)-phthalazinone compounds which act as PARP inhibitors. The compounds have the general formula:

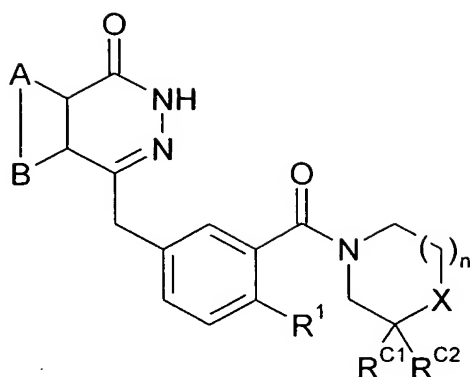


- where A and B together represent an optionally substituted, fused aromatic ring and where R_C is represented by $-L-R_L$. A large number of examples are of the formula:



where R represent one or more optional substituents.

- The present inventors have now discovered that compounds where R is of a certain nature exhibit surprising levels of inhibition of the activity of PARP, and/or of potentiation of tumour cells to radiotherapy and various chemotherapies.
- Accordingly, the first aspect of the present invention provides a compound of the formula (I):

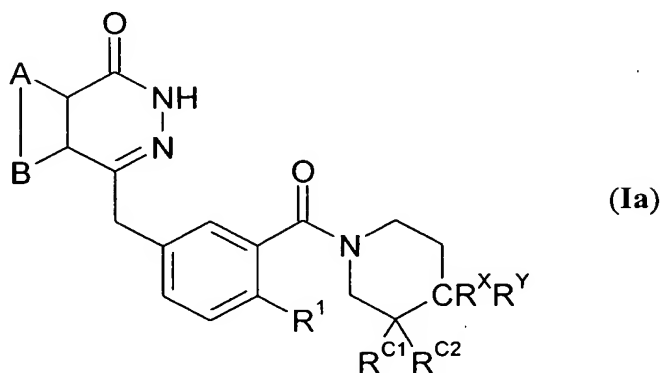


and isomers, salts, solvates, chemically protected forms, and prodrugs thereof

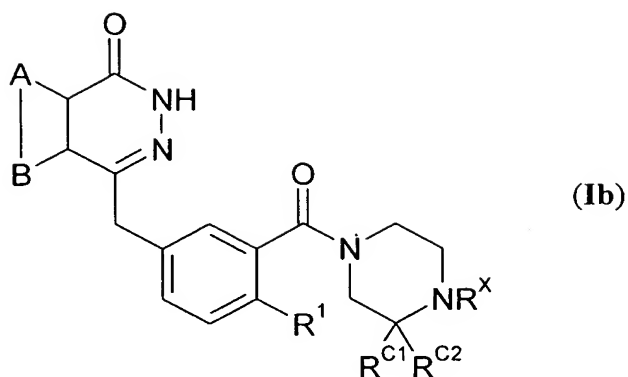
wherein:

- 5 A and B together represent an optionally substituted, fused aromatic ring;
X can be NR^{X} or $\text{CR}^{\text{X}}\text{R}^{\text{Y}}$;
if $\text{X} = \text{NR}^{\text{X}}$ then n is 1 or 2 and if $\text{X} = \text{CR}^{\text{X}}\text{R}^{\text{Y}}$ then n is 1;
 R^{X} is selected from the group consisting of H, optionally substituted
- 10 C_{1-20} alkyl, C_{5-20} aryl, C_{3-20} heterocyclyl, amido, thioamido, ester, acyl, and sulfonyl groups;
 R^{Y} is selected from H, hydroxy, amino;
or R^{X} and R^{Y} may together form a spiro- C_{3-7} cycloalkyl or heterocyclyl group;
- 15 $\text{R}^{\text{C}1}$ and $\text{R}^{\text{C}2}$ are both hydrogen, or when X is $\text{CR}^{\text{X}}\text{R}^{\text{Y}}$, $\text{R}^{\text{C}1}$, $\text{R}^{\text{C}2}$, R^{X} and R^{Y} , together with the carbon atoms to which they are attached, may form an optionally substituted fused aromatic ring; and
 R^1 is selected from H and halo.

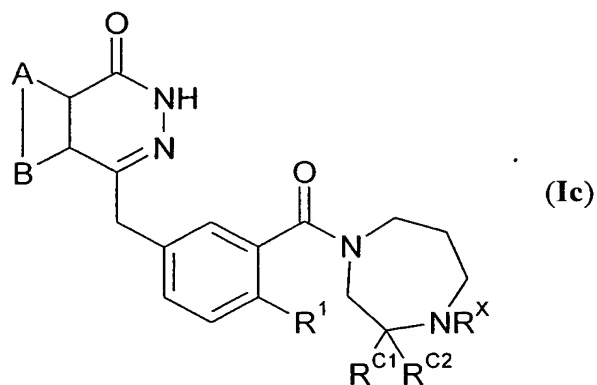
- 20 Therefore, if X is $\text{CR}^{\text{X}}\text{R}^{\text{Y}}$, then n is 1, the compound is of formula (Ia):



If X is NR^{X} , and n is 1, the compound is of formula (Ib):



If X is NR^x, and n is 2, the compound is of formula (Ic):



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A second aspect of the present invention provides a pharmaceutical composition comprising a compound of the first aspect and a pharmaceutically acceptable carrier or diluent.

- 10 A third aspect of the present invention provides a compound of the first aspect for use in a method of treatment of the human or animal body.

- A fourth aspect of the present invention provides the use of a
 15 compound as defined in the first aspect of the invention in the preparation of a medicament for inhibiting the activity of PARP.

- Further aspects of the invention provide the use of a compound as defined in the first aspect of the invention in the preparation of a
 20 medicament for the treatment of: vascular disease; septic shock; ischaemic injury; neurotoxicity; haemorrhagic shock; viral infection; or diseases ameliorated by the inhibition of the activity of PARP.

Another further aspect of the invention provides for the use of a compound as defined in the first aspect of the invention in the preparation of a medicament for use as an adjunct in cancer therapy or for potentiating tumour cells for treatment with ionizing radiation or
5 chemotherapeutic agents.

Other further aspects of the invention provide for the treatment of disease ameliorated by the inhibition of PARP, comprising administering to a subject in need of treatment a therapeutically-effective amount of a compound as defined in the first aspect,
10 preferably in the form of a pharmaceutical composition and the treatment of cancer, comprising administering to a subject in need of treatment a therapeutically-effective amount of a compound as defined in the first aspect in combination, preferably in the form of a
15 pharmaceutical composition, simultaneously or sequentially with ionizing radiation or chemotherapeutic agents.

In further aspects of the present invention, the compounds may be used in the preparation of a medicament for the treatment of cancer which
20 is deficient in Homologous Recombination (HR) dependent DNA DSB repair activity, or in the treatment of a patient of a cancer which is deficient in HR dependent DNA DSB repair activity, comprising administering to said patient a therapeutically-effective amount of the compound.

25 The HR dependent DNA DSB repair pathway repairs double-strand breaks (DSBs) in DNA via homologous mechanisms to reform a continuous DNA helix (K.K. Khanna and S.P. Jackson, Nat. Genet. 27(3): 247-254 (2001)). The components of the HR dependent DNA DSB repair pathway
30 include, but are not limited to, ATM (NM_000051), RAD51 (NM_002875), RAD51L1 (NM_002877), RAD51C (NM_002876), RAD51L3 (NM_002878), DMC1 (NM_007068), XRCC2 (NM_005431), XRCC3 (NM_005432), RAD52 (NM_002879), RAD54L (NM_003579), RAD54B (NM_012415), BRCA1 (NM_007295), BRCA2 (NM_000059), RAD50 (NM_005732), MRE11A (NM_005590) and NBS1
35 (NM_002485). Other proteins involved in the HR dependent DNA DSB repair pathway include regulatory factors such as EMSY (Hughes-Davies, *et al.*, *Cell*, **115**, pp523-535). HR components are also described in Wood, *et al.*, *Science*, **291**, 1284-1289 (2001).

A cancer which is deficient in HR dependent DNA DSB repair may comprise or consist of one or more cancer cells which have a reduced or abrogated ability to repair DNA DSBs through that pathway, relative to normal cells i.e. the activity of the HR dependent DNA DSB repair pathway may be reduced or abolished in the one or more cancer cells.

The activity of one or more components of the HR dependent DNA DSB repair pathway may be abolished in the one or more cancer cells of an individual having a cancer which is deficient in HR dependent DNA DSB repair. Components of the HR dependent DNA DSB repair pathway are well characterised in the art (see for example, Wood, et al., *Science*, **291**, 1284-1289 (2001)) and include the components listed above.

In some preferred embodiments, the cancer cells may have a BRCA1 and/or a BRCA2 deficient phenotype i.e. BRCA1 and/or BRCA2 activity is reduced or abolished in the cancer cells. Cancer cells with this phenotype may be deficient in BRCA1 and/or BRCA2, i.e. expression and/or activity of BRCA1 and/or BRCA2 may be reduced or abolished in the cancer cells, for example by means of mutation or polymorphism in the encoding nucleic acid, or by means of amplification, mutation or polymorphism in a gene encoding a regulatory factor, for example the EMSY gene which encodes a BRCA2 regulatory factor (Hughes-Davies, et al., *Cell*, **115**, 523-535).

BRCA1 and BRCA2 are known tumour suppressors whose wild-type alleles are frequently lost in tumours of heterozygous carriers (Jasin M., *Oncogene*, **21(58)**, 8981-93 (2002); Tutt, et al., *Trends Mol Med.*, **8(12)**, 571-6, (2002)). The association of BRCA1 and/or BRCA2 mutations with breast cancer is well-characterised in the art (Radice, P.J., *Exp Clin Cancer Res.*, **21(3 Suppl)**, 9-12 (2002)). Amplification of the EMSY gene, which encodes a BRCA2 binding factor, is also known to be associated with breast and ovarian cancer.

Carriers of mutations in BRCA1 and/or BRCA2 are also at elevated risk of cancer of the ovary, prostate and pancreas.

In some preferred embodiments, the individual is heterozygous for one or more variations, such as mutations and polymorphisms, in BRCA1 and/or BRCA2 or a regulator thereof. The detection of variation in

BRCA1 and BRCA2 is well-known in the art and is described, for example in EP 699 754, EP 705 903, Neuhausen, S.L. and Ostrander, E.A., *Genet. Test*, **1**, 75-83 (1992); Chappnis, P.O. and Foulkes, W.D., *Cancer Treat Res*, **107**, 29-59 (2002); Janatova M., et al., *Neoplasma*, **50(4)**, 246-50 (2003); Jancarkova, N., *Ceska Gynkol.*, **68(1)**, 11-6 (2003)).

Determination of amplification of the BRCA2 binding factor EMSY is described in Hughes-Davies, et al., *Cell*, **115**, 523-535).

Mutations and polymorphisms associated with cancer may be detected at the nucleic acid level by detecting the presence of a variant nucleic acid sequenc or at the protein level by detecting the presence of a variant (i.e. a mutant or allelic variant) polypeptide.

Definitions

The term "aromatic ring" is used herein in the conventional sense to refer to a cyclic aromatic structure, that is, a cyclic structure having delocalised π -electron orbitals.

The aromatic ring fused to the main core, i.e. that formed by -A-B-, may bear further fused aromatic rings (resulting in, e.g. naphthyl or anthracenyl groups). The aromatic ring(s) may comprise solely carbon atoms, or may comprise carbon atoms and one or more heteroatoms, including but not limited to, nitrogen, oxygen, and sulfur atoms. The aromatic ring(s) preferably have five or six ring atoms.

The aromatic ring(s) may optionally be substituted. If a substituent itself comprises an aryl group, this aryl group is not considered to be a part of the aryl group to which it is attached. For example, the group biphenyl is considered herein to be a phenyl group (an aryl group comprising a single aromatic ring) substituted with a phenyl group. Similarly, the group benzylphenyl is considered to be a phenyl group (an aryl group comprising a single aromatic ring) substituted with a benzyl group.

In one group of preferred embodiments, the aromatic group comprises a single aromatic ring, which has five or six ring atoms, which ring atoms are selected from carbon, nitrogen, oxygen, and sulfur, and which ring is optionally substituted. Examples of these groups

include, but are not limited to, benzene, pyrazine, pyrrole, thiazole, isoxazole, and oxazole. 2-Pyrone can also be considered to be an aromatic ring, but is less preferred.

- 5 If the aromatic ring has six atoms, then preferably at least four, or even five or all, of the ring atoms are carbon. The other ring atoms are selected from nitrogen, oxygen and sulphur, with nitrogen and oxygen being preferred. Suitable groups include a ring with: no hetero atoms (benzene); one nitrogen ring atom (pyridine); two
10 nitrogen ring atoms (pyrazine, pyrimidine and pyridazine); one oxygen ring atom (pyrone); and one oxygen and one nitrogen ring atom (oxazine).

If the aromatic ring has five ring atoms, then preferably at least
15 three of the ring atoms are carbon. The remaining ring atoms are selected from nitrogen, oxygen and sulphur. Suitable rings include a ring with: one nitrogen ring atom (pyrrole); two nitrogen ring atoms (imidazole, pyrazole); one oxygen ring atom (furan); one sulphur ring atom (thiophene); one nitrogen and one sulphur ring atom (isothiazole, thiazole); and one nitrogen and one oxygen ring atom (isoxazole or
20 oxazole).

The aromatic ring may bear one or more substituent groups at any available ring position. These substituents are selected from halo,
25 nitro, hydroxy, ether, thiol, thioether, amino, C₁₋₇ alkyl, C₃₋₂₀ heterocyclyl and C₅₋₂₀ aryl. The aromatic ring may also bear one or more substituent groups which together form a ring. In particular these may be of formula $-(CH_2)_m-$ or $-O-(CH_2)_p-O-$, where m is 2, 3, 4 or 5 and p is 1, 2 or 3.

30 Alkyl: The term "alkyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 1 to 20 carbon atoms (unless otherwise specified), which may be aliphatic or alicyclic, and which
35 may be saturated or unsaturated (e.g. partially unsaturated, fully unsaturated). Thus, the term "alkyl" includes the sub-classes alkenyl, alkynyl, cycloalkyl, cycloalkenyl, cycloalkynyl, etc., discussed below.

In the context of alkyl groups, the prefixes (e.g. C₁₋₄, C₁₋₇, C₁₋₂₀, C₂₋₇, C₃₋₇, etc.) denote the number of carbon atoms, or range of number of carbon atoms. For example, the term "C₁₋₄ alkyl", as used herein, pertains to an alkyl group having from 1 to 4 carbon atoms. Examples of groups of alkyl groups include C₁₋₄ alkyl ("lower alkyl"), C₁₋₇ alkyl, and C₁₋₂₀ alkyl. Note that the first prefix may vary according to other limitations; for example, for unsaturated alkyl groups, the first prefix must be at least 2; for cyclic alkyl groups, the first prefix must be at least 3; etc.

Examples of (unsubstituted) saturated alkyl groups include, but are not limited to, methyl (C₁), ethyl (C₂), propyl (C₃), butyl (C₄), pentyl (C₅), hexyl (C₆), heptyl (C₇), octyl (C₈), nonyl (C₉), decyl (C₁₀), undecyl (C₁₁), dodecyl (C₁₂), tridecyl (C₁₃), tetradecyl (C₁₄), pentadecyl (C₁₅), and eicododecyl (C₂₀).

Examples of (unsubstituted) saturated linear alkyl groups include, but are not limited to, methyl (C₁), ethyl (C₂), n-propyl (C₃), n-butyl (C₄), n-pentyl (amyl) (C₅), n-hexyl (C₆), and n-heptyl (C₇).

Examples of (unsubstituted) saturated branched alkyl groups include iso-propyl (C₃), iso-butyl (C₄), sec-butyl (C₄), tert-butyl (C₄), iso-pentyl (C₅), and neo-pentyl (C₅).

Alkenyl: The term "alkenyl", as used herein, pertains to an alkyl group having one or more carbon-carbon double bonds. Examples of groups of alkenyl groups include C₂₋₄ alkenyl, C₂₋₇ alkenyl, C₂₋₂₀ alkenyl.

Examples of (unsubstituted) unsaturated alkenyl groups include, but are not limited to, ethenyl (vinyl, -CH=CH₂), 1-propenyl (-CH=CH-CH₃), 2-propenyl (allyl, -CH=CH-CH₂), isopropenyl (1-methylvinyl, -C(CH₃)=CH₂), butenyl (C₄), pentenyl (C₅), and hexenyl (C₆).

Alkynyl: The term "alkynyl", as used herein, pertains to an alkyl group having one or more carbon-carbon triple bonds. Examples of groups of alkynyl groups include C₂₋₄ alkynyl, C₂₋₇ alkynyl, C₂₋₂₀ alkynyl.

Examples of (unsubstituted) unsaturated alkynyl groups include, but are not limited to, ethynyl (ethynyl, -C≡CH) and 2-propynyl

(propargyl, $-\text{CH}_2-\text{C}\equiv\text{CH}$).

Cycloalkyl: The term "cycloalkyl", as used herein, pertains to an alkyl group which is also a cyclyl group; that is, a monovalent moiety obtained by removing a hydrogen atom from an alicyclic ring atom of a carbocyclic ring of a carbocyclic compound, which carbocyclic ring may be saturated or unsaturated (e.g. partially unsaturated, fully unsaturated), which moiety has from 3 to 20 carbon atoms (unless otherwise specified), including from 3 to 20 ring atoms. Thus, the term "cycloalkyl" includes the sub-classes cycloalkenyl and cycloalkynyl. Preferably, each ring has from 3 to 7 ring atoms. Examples of groups of cycloalkyl groups include C_{3-20} cycloalkyl, C_{3-15} cycloalkyl, C_{3-10} cycloalkyl, C_{3-7} cycloalkyl.

Examples of cycloalkyl groups include, but are not limited to, those derived from:

saturated monocyclic hydrocarbon compounds:

cyclopropane (C_3), cyclobutane (C_4), cyclopentane (C_5), cyclohexane (C_6), cycloheptane (C_7), methylcyclopropane (C_4), dimethylcyclopropane (C_5), methylcyclobutane (C_5), dimethylcyclobutane (C_6), methylcyclopentane (C_6), dimethylcyclopentane (C_7), methylcyclohexane (C_7), dimethylcyclohexane (C_8), menthane (C_{10});

unsaturated monocyclic hydrocarbon compounds:

cyclopropene (C_3), cyclobutene (C_4), cyclopentene (C_5), cyclohexene (C_6), methylcyclopropene (C_4), dimethylcyclopropene (C_5), methylcyclobutene (C_5), dimethylcyclobutene (C_6), methylcyclopentene (C_6), dimethylcyclopentene (C_7), methylcyclohexene (C_7), dimethylcyclohexene (C_8);

saturated polycyclic hydrocarbon compounds:

thujane (C_{10}), carane (C_{10}), pinane (C_{10}), bornane (C_{10}), norcarane (C_7), norpinane (C_7), norbornane (C_7), adamantane (C_{10}), decalin (decahydronaphthalene) (C_{10});

unsaturated polycyclic hydrocarbon compounds:

camphene (C_{10}), limonene (C_{10}), pinene (C_{10});
polycyclic hydrocarbon compounds having an aromatic ring:
indene (C_9), indane (e.g., 2,3-dihydro-1H-indene) (C_9), tetraline (1,2,3,4-tetrahydronaphthalene) (C_{10}), acenaphthene (C_{12}), fluorene (C_{13}), phenalene (C_{13}), acephenanthrene (C_{15}), aceanthrene (C_{16}), cholanthrene (C_{20}).

Heterocyclyl: The term "heterocyclyl", as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a ring atom of a heterocyclic compound, which moiety has from 3 to 20 ring atoms (unless otherwise specified), of which from 1 to 10 are ring heteroatoms. Preferably, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms.

In this context, the prefixes (e.g. C₃₋₂₀, C₃₋₇, C₅₋₆, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For example, the term "C₅₋₆heterocyclyl", as used herein, pertains to a heterocyclyl group having 5 or 6 ring atoms. Examples of groups of heterocyclyl groups include C₃₋₂₀ heterocyclyl, C₅₋₂₀ heterocyclyl, C₃₋₁₅ heterocyclyl, C₅₋₁₅ heterocyclyl, C₃₋₁₂ heterocyclyl, C₅₋₁₂ heterocyclyl, C₃₋₁₀ heterocyclyl, C₅₋₁₀ heterocyclyl, C₃₋₇ heterocyclyl, C₅₋₇ heterocyclyl, and C₅₋₆ heterocyclyl.

Examples of monocyclic heterocyclyl groups include, but are not limited to, those derived from:

N₁: aziridine (C₃), azetidine (C₄), pyrrolidine (tetrahydropyrrole) (C₅), pyrroline (e.g., 3-pyrroline, 2,5-dihydropyrrole) (C₅), 2H-pyrrole or 3H-pyrrole (isopyrrole, isoazole) (C₅), piperidine (C₆), dihydropyridine (C₆), tetrahydropyridine (C₆), azepine (C₇);

O₁: oxirane (C₃), oxetane (C₄), oxolane (tetrahydrofuran) (C₅), oxole (dihydrofuran) (C₅), oxane (tetrahydropyran) (C₆), dihydropyran (C₆), pyran (C₆), oxepin (C₇);

S₁: thiirane (C₃), thietane (C₄), thiolane (tetrahydrothiophene) (C₅), thiane (tetrahydrothiopyran) (C₆), thiepane (C₇);

O₂: dioxolane (C₅), dioxane (C₆), and dioxepane (C₇);

O₃: trioxane (C₆);

N₂: imidazolidine (C₅), pyrazolidine (diazolidine) (C₅), imidazoline (C₅), pyrazoline (dihydropyrazole) (C₅), piperazine (C₆);

N_1O_1 : tetrahydrooxazole (C_5), dihydrooxazole (C_5), tetrahydroisoxazole (C_5), dihydroisoxazole (C_5), morpholine (C_6), tetrahydrooxazine (C_6), dihydrooxazine (C_6), oxazine (C_6);

5 N_1S_1 : thiazoline (C_5), thiazolidine (C_5), thiomorpholine (C_6);

N_2O_1 : oxadiazine (C_6);

O_1S_1 : oxathiole (C_5) and oxathiane (thioxane) (C_6); and,

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$N_1O_1S_1$: oxathiazine (C_6).

Examples of substituted (non-aromatic) monocyclic heterocyclyl groups include those derived from saccharides, in cyclic form, for example, 15 furanoses (C_5), such as arabinofuranose, lyxofuranose, ribofuranose, and xylofuranse, and pyranoses (C_6), such as allopyranose, altropyranose, glucopyranose, mannopyranose, gulopyranose, idopyranose, galactopyranose, and talopyranose.

20 Spiro- C_{3-7} cycloalkyl or heterocyclyl: The term "spiro C_{3-7} cycloalkyl or heterocyclyl" as used herein, refers to a C_{3-7} cycloalkyl or C_{3-7} heterocyclyl ring joined to another ring by a single atom common to both rings.

25 C_{5-20} aryl: The term " C_{5-20} aryl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of a C_{5-20} aromatic compound, said compound having one ring, or two or more rings (e.g., fused), and having from 5 to 20 ring atoms, and wherein at least one of said ring(s) is an aromatic ring. Preferably, 30 each ring has from 5 to 7 ring atoms.

The ring atoms may be all carbon atoms, as in "carboaryl groups" in which case the group may conveniently be referred to as a " C_{5-20} carboaryl" group.

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Examples of C_{5-20} aryl groups which do not have ring heteroatoms (i.e. C_{5-20} carboaryl groups) include, but are not limited to, those derived from benzene (i.e. phenyl) (C_6), naphthalene (C_{10}), anthracene (C_{14}), phenanthrene (C_{14}), and pyrene (C_{16}).

Alternatively, the ring atoms may include one or more heteroatoms, including but not limited to oxygen, nitrogen, and sulfur, as in "heteroaryl groups". In this case, the group may conveniently be referred to as a "C₅₋₂₀ heteroaryl" group, wherein "C₅₋₂₀" denotes ring atoms, whether carbon atoms or heteroatoms. Preferably, each ring has from 5 to 7 ring atoms, of which from 0 to 4 are ring heteroatoms.

Examples of C₅₋₂₀ heteroaryl groups include, but are not limited to, C₅ heteroaryl groups derived from furan (oxole), thiophene (thiole), pyrrole (azole), imidazole (1,3-diazole), pyrazole (1,2-diazole), triazole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, tetrazole and oxatriazole; and C₆ heteroaryl groups derived from isoxazine, pyridine (azine), pyridazine (1,2-diazine), pyrimidine (1,3-diazine; e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine) and triazine.

The heteroaryl group may be bonded via a carbon or hetero ring atom.

Examples of C₅₋₂₀ heteroaryl groups which comprise fused rings, include, but are not limited to, C₉ heteroaryl groups derived from benzofuran, isobenzofuran, benzothiophene, indole, isoindole; C₁₀ heteroaryl groups derived from quinoline, isoquinoline, benzodiazine, pyridopyridine; C₁₄ heteroaryl groups derived from acridine and xanthene.

The above alkyl, heterocyclyl, and aryl groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below.

Halo: -F, -Cl, -Br, and -I.

Hydroxy: -OH.

Ether: -OR, wherein R is an ether substituent, for example, a C₁₋₇ alkyl group (also referred to as a C₁₋₇ alkoxy group), a C₃₋₂₀ heterocyclyl group (also referred to as a C₃₋₂₀ heterocyclyloxy group), or a C₅₋₂₀ aryl group (also referred to as a C₅₋₂₀ aryloxy group), preferably a C₁₋₇ alkyl group.

Nitro: -NO_2 .

Cyano (nitrile, carbonitrile): -CN .

5

Acyl (keto): -C(=O)R , wherein R is an acyl substituent, for example, H, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylacyl or C_{1-7} alkanoyl), a C_{3-20} heterocyclyl group (also referred to as C_{3-20} heterocyclylacyl), or a C_{5-20} aryl group (also referred to as C_{5-20} arylacyl), preferably a C_{1-7} alkyl group. Examples of acyl groups include, but are not limited to, -C(=O)CH_3 (acetyl), $\text{-C(=O)CH}_2\text{CH}_3$ (propionyl), $\text{-C(=O)C(CH}_3)_3$ (butyryl), and -C(=O)Ph (benzoyl, phenone).

10

Carboxy (carboxylic acid): -COOH .

15

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=O)OR , wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, -C(=O)OCH_3 , $\text{-C(=O)OCH}_2\text{CH}_3$, $\text{-C(=O)OC(CH}_3)_3$, and -C(=O)OPh .

20

Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $\text{-C(=O)NR}^1\text{R}^2$, wherein R^1 and R^2 are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, -C(=O)NH_2 , -C(=O)NHCH_3 , $\text{-C(=O)N(CH}_3)_2$, $\text{-C(=O)NHCH}_2\text{CH}_3$, and $\text{-C(=O)N(CH}_2\text{CH}_3)_2$, as well as amido groups in which R^1 and R^2 , together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinylcarbonyl.

25

30

Amino: $\text{-NR}^1\text{R}^2$, wherein R^1 and R^2 are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di- C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group, or, in the case of a "cyclic" amino group, R^1 and R^2 , taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Examples of amino groups include, but are not limited to, -NH_2 , -NHCH_3 , $\text{-NHCH(CH}_3)_2$, $\text{-N(CH}_3)_2$, $\text{-N(CH}_2\text{CH}_3)_2$, and -NHPh . Examples of cyclic amino groups include, but are not limited to, aziridinyl,

35

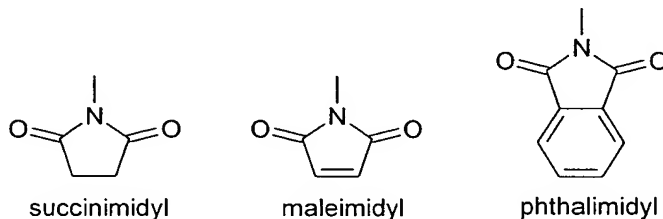
azetidiny, pyrrolidiny, piperidino, piperaziny, perhydrodiazepiny, morpholino, and thiomorpholino. The cyclic amino groups may be substituted on their ring by any of the substituents defined here, for example carboxy, carboxylate and amido.

5

Acylamido (acylamino): $-NR^1C(=O)R^2$, wherein R^1 is an amide substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group, most preferably H, and R^2 is an acyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20}

10 heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group.

Examples of acylamide groups include, but are not limited to, $-NHC(=O)CH_3$, $-NHC(=O)CH_2CH_3$, and $-NHC(=O)Ph$. R^1 and R^2 may together form a cyclic structure, as in, for example, succinimidyl, maleimidyl, and phthalimidyl:



15

Ureido: $-N(R^1)CONR^2R^3$ wherein R^2 and R^3 are independently amino substituents, as defined for amino groups, and R^1 is a ureido substituent, for example, hydrogen, a C_{1-7} alkyl group, a

20 C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of ureido groups include, but are not limited to, $-NHCONH_2$, $-NHCONHMe$, $-NHCONHEt$, $-NHCONMe_2$, $-NHCONEt_2$, $-NMeCONH_2$, $-NMeCONHMe$, $-NMeCONHEt$, $-NMeCONMe_2$, $-NMeCONEt_2$ and $-NHC(=O)NHPh$.

25

Acyloxy (reverse ester): $-OC(=O)R$, wherein R is an acyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of acyloxy groups include, but are not limited to, $-OC(=O)CH_3$ (acetoxy), $-OC(=O)CH_2CH_3$, $-OC(=O)C(CH_3)_3$, $-OC(=O)Ph$, $-OC(=O)C_6H_4F$, and $-OC(=O)CH_2Ph$.

30

Thiol : $-SH$.

Thioether (sulfide): $-SR$, wherein R is a thioether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkylthio group),

35

a C₃₋₂₀ heterocyclyl group, or a C₅₋₂₀ aryl group, preferably a C₁₋₇ alkyl group. Examples of C₁₋₇ alkylthio groups include, but are not limited to, -SCH₃ and -SCH₂CH₃.

- 5 Sulfoxide (sulfinyl): -S(=O)R, wherein R is a sulfoxide substituent, for example, a C₁₋₇ alkyl group, a C₃₋₂₀ heterocyclyl group, or a C₅₋₂₀ aryl group, preferably a C₁₋₇ alkyl group. Examples of sulfoxide groups include, but are not limited to, -S(=O)CH₃ and -S(=O)CH₂CH₃.
- 10 Sulfonyl (sulfone): -S(=O)₂R, wherein R is a sulfone substituent, for example, a C₁₋₇ alkyl group, a C₃₋₂₀ heterocyclyl group, or a C₅₋₂₀ aryl group, preferably a C₁₋₇ alkyl group. Examples of sulfone groups include, but are not limited to, -S(=O)₂CH₃ (methanesulfonyl, mesyl), -S(=O)₂CF₃, -S(=O)₂CH₂CH₃, and 4-methylphenylsulfonyl (tosyl).
- 15 Thioamido (thiocarbamyl): -C(=S)NR¹R², wherein R¹ and R² are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, -C(=S)NH₂, -C(=S)NHCH₃, -C(=S)N(CH₃)₂, and -C(=S)NHCH₂CH₃.
- 20 Sulfonamino: -NR¹S(=O)₂R, wherein R¹ is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a C₁₋₇alkyl group, a C₃₋₂₀heterocyclyl group, or a C₅₋₂₀aryl group, preferably a C₁₋₇alkyl group. Examples of sulfonamino groups
- 25 include, but are not limited to, -NHS(=O)₂CH₃, -NHS(=O)₂Ph and -N(CH₃)S(=O)₂C₆H₅.

As mentioned above, the groups that form the above listed substituent groups, e.g. C₁₋₇ alkyl, C₃₋₂₀ heterocyclyl and C₅₋₂₀ aryl, may themselves

30 be substituted. Thus, the above definitions cover substituent groups which are substituted.

Brief Description of Figures

Figure 1 shows clonogenic survival curves of cells exposed to

35 compound (4) of the present invention.

Figure 1A shows Brcal wild type (11CO:■), heterozygous (Cre6:▲) and deficient (Cre10:●) ES cells under continuous exposure to compound 4. Error bars represent standard errors of the mean.

Figure 1B shows Brca2 wild type (D3:■), heterozygous (Cre6:▲) and deficient (Cre24:●) ES cells under continuous exposure to compound 4. Error bars represent standard errors of the mean.

5

Figure 2 shows an analysis of the effects of a compound of the invention (4) in another cell line lacking BRCA2 function in comparison to a BRCA2 complemented line. Data shown is clonogenic survival of Brca2 deficient (V-C8:■) and complemented (V-C8 BAC+:▲) cells under continuous exposure to compound 4 at varying concentrations.

10

Further Preferences

The following preferences can apply to each aspect of the present invention, where applicable.

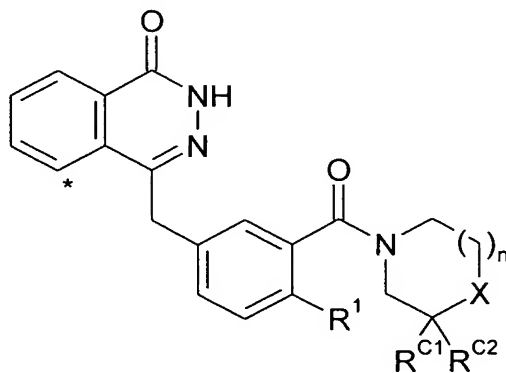
15

In the present invention, the fused aromatic ring(s) represented by -A-B- preferably consist of solely carbon ring atoms, and thus may be benzene, naphthalene, and is more preferably benzene. As described above, these rings may be substituted, but in some embodiments are preferably unsubstituted.

20

If the fused aromatic ring represented by -A-B- bears a substituent group, it is preferably attached to the atom which itself is attached to the central ring meta- to the carbonyl group. Thus, if the fused aromatic ring is a benzene ring, the preferred place of substitution is shown in the formula below by *:

25



which is usually termed the 5-position of the phthalazinone moiety.

30

R¹ is preferably selected from H, Cl and F, and is more preferably F.

It is preferred that R^{C1} and R^{C2} are both hydrogen.

When n is 2, X is NR^X . In these embodiments, R^X is preferably selected
5 from the group consisting of: H; optionally substituted C_{1-20} alkyl;
optionally substituted C_{5-20} aryl; optionally substituted ester groups,
wherein the ester substituent is preferably C_{1-20} alkyl; optionally
substituted acyl groups; optionally substituted amido groups;
optionally substituted thioamido groups; and optionally substituted
10 sulfonyl groups. R^X is more preferably selected from the group
consisting of: H; optionally substituted C_{1-20} alkyl; optionally
substituted C_{5-20} aryl; and optionally substituted ester groups, wherein
the ester substituent is preferably C_{1-20} alkyl.

15 When n is 1, X may be NR^X or CR^XCR^Y .

In embodiments where X is NR^X , R^X is preferably selected from the group
consisting of: H; optionally substituted C_{1-20} alkyl; optionally
substituted C_{5-20} aryl; optionally substituted acyl; optionally
20 substituted sulfonyl; optionally substituted amido; and optionally
substituted thioamido groups.

In embodiments where X is CR^XCR^Y , R^Y is preferably H. R^X is preferably
selected from the group consisting of: H; optionally substituted C_{1-20}
25 alkyl; optionally substituted C_{5-20} aryl; optionally substituted C_{3-20}
heterocyclyl; optionally substituted acyl, wherein the acyl
substituent is preferably selected from C_{5-20} aryl and C_{3-20} heterocyclyl
(e.g. piperazinyl); optionally substituted amido, wherein the amino
groups are preferably selected from H and C_{1-20} alkyl or together with
30 the nitrogen atom, form a C_{5-20} heterocyclic group; and optionally
substituted ester groups, wherein the ester substituent is preferably
selected from C_{1-20} alkyl groups.

Particularly preferred compounds include: 1, 2, 3, 4, 10, 21, 74, 97,
35 152, 153, 163, 167, 169, 173, 185, 232, 233, 250, 251, 252, 260 and
263.

Where appropriate, the above preferences may be taken in combination
with each other.

Includes Other Forms

Included in the above are the well known ionic, salt, solvate, and protected forms of these substituents. For example, a reference to carboxylic acid (-COOH) also includes the anionic (carboxylate) form (-COO⁻), a salt or solvate thereof, as well as conventional protected forms. Similarly, a reference to an amino group includes the protonated form (-N⁺HR¹R²), a salt or solvate of the amino group, for example, a hydrochloride salt, as well as conventional protected forms of an amino group. Similarly, a reference to a hydroxyl group also includes the anionic form (-O⁻), a salt or solvate thereof, as well as conventional protected forms of a hydroxyl group.

Isomers, Salts, Solvates, Protected Forms, and Prodrugs

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diastereomeric, epimeric, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, *cis*- and *trans*-forms; *E*- and *Z*-forms; *c*-, *t*-, and *r*-forms; *endo*- and *exo*-forms; *R*-, *S*-, and *meso*-forms; *D*- and *L*-forms; *d*- and *l*-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

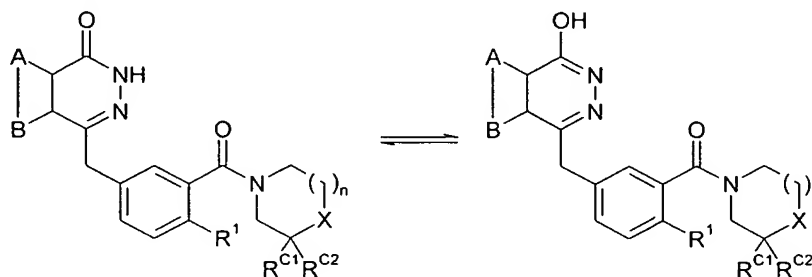
If the compound is in crystalline form, it may exist in a number of different polymorphic forms.

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers", as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, -OCH₃, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, -CH₂OH. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g., C₁₋₇ alkyl includes *n*-propyl and *iso*-propyl; butyl

includes *n*-, *iso*-, *sec*-, and *tert*-butyl; methoxyphenyl includes *ortho*-, *meta*-, and *para*-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol, imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, *N*-nitroso/hydroxyazo, and nitro/aci-nitro.

- 10 Particularly relevant to the present invention is the tautomeric pair illustrated below:



- Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^1H , ^2H (D), and ^3H (T); C may be in any isotopic form, including ^{12}C , ^{13}C , and ^{14}C ; O may be in any isotopic form, including ^{16}O and ^{18}O ; and the like.

- Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g. fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

- Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate, and protected forms of thereof, for example, as discussed below, as well as its different polymorphic forms.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, et al., "Pharmaceutically Acceptable Salts", *J. Pharm. Sci.*, **66**, 1-19 (1977).

For example, if the compound is anionic, or has a functional group which may be anionic (e.g., $-\text{COOH}$ may be $-\text{COO}^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+ , alkaline earth cations such as Ca^{2+} and Mg^{2+} , and other cations such as Al^{3+} . Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH_4^+) and substituted ammonium ions (e.g., NH_3R^+ , NH_2R_2^+ , NHR_3^+ , NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $\text{N}(\text{CH}_3)_4^+$.

If the compound is cationic, or has a functional group which may be cationic (e.g., $-\text{NH}_2$ may be $-\text{NH}_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous. Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: acetic, propionic, succinic, glycolic, stearic, palmitic, lactic, malic, pantoic, tartaric, citric, gluconic, ascorbic, maleic, hydroxymaleic, phenylacetic, glutamic, aspartic, benzoic, cinnamic, pyruvic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethanesulfonic, ethane disulfonic, oxalic, isethionic, valeric, and gluconic. Examples of suitable polymeric anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form," as used herein, pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions, that is, are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, "Protective Groups in Organic Synthesis" (T. Green and P. Wuts; 3rd Edition; John Wiley and Sons, 1999).

For example, a hydroxy group may be protected as an ether ($-OR$) or an ester ($-OC(=O)R$), for example, as: a *t*-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or *t*-butyldimethylsilyl ether; or an acetyl ester ($-OC(=O)CH_3$, $-OAc$).

For example, an aldehyde or ketone group may be protected as an acetal or ketal, respectively, in which the carbonyl group ($>C=O$) is converted to a diether ($>C(OR)_2$), by reaction with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.

For example, an amine group may be protected, for example, as an amide or a urethane, for example, as: a methyl amide ($-NHCO-CH_3$); a benzyloxy amide ($-NHCO-OCH_2C_6H_5$, $-NH-Cbz$); as a *t*-butoxy amide ($-NHCO-OC(CH_3)_3$, $-NH-Boc$); a 2-biphenyl-2-propoxy amide ($-NHCO-OC(CH_3)_2C_6H_4C_6H_5$, $-NH-Bpoc$), as a 9-fluorenylmethoxy amide ($-NH-Fmoc$), as a 6-

nitroveratryloxy amide (-NH-Nvoc), as a 2-trimethylsilylethyloxy amide (-NH-Teoc), as a 2,2,2-trichloroethyloxy amide (-NH-Troc), as an allyloxy amide (-NH-Alloc), as a 2(-phenylsulphonyl)ethyloxy amide (-NH-Psec); or, in suitable cases, as an N-oxide (>NO•).

5

For example, a carboxylic acid group may be protected as an ester for example, as: an C₁₋₇ alkyl ester (e.g. a methyl ester; a *t*-butyl ester); a C₁₋₇ haloalkyl ester (e.g. a C₁₋₇ trihaloalkyl ester); a triC₁₋₇ alkylsilyl-C₁₋₇ alkyl ester; or a C₅₋₂₀ aryl-C₁₋₇ alkyl ester (e.g. a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

For example, a thiol group may be protected as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether (-SCH₂NHC(=O)CH₃).

It may be convenient or desirable to prepare, purify, and/or handle the active compound in the form of a prodrug. The term "prodrug", as used herein, pertains to a compound which, when metabolised (e.g. *in vivo*), yields the desired active compound. Typically, the prodrug is inactive, or less active than the active compound, but may provide advantageous handling, administration, or metabolic properties.

For example, some prodrugs are esters of the active compound (e.g. a physiologically acceptable metabolically labile ester). During metabolism, the ester group (-C(=O)OR) is cleaved to yield the active drug. Such esters may be formed by esterification, for example, of any of the carboxylic acid groups (-C(=O)OH) in the parent compound, with, where appropriate, prior protection of any other reactive groups present in the parent compound, followed by deprotection if required.

Examples of such metabolically labile esters include those wherein R is C₁₋₂₀ alkyl (e.g. -Me, -Et); C₁₋₇ aminoalkyl (e.g. aminoethyl; 2-(*N,N*-diethylamino)ethyl; 2-(4-morpholino)ethyl); and acyloxy-C₁₋₇ alkyl (e.g. acyloxymethyl; acyloxyethyl; e.g. pivaloyloxymethyl; acetoxymethyl; 1-acetoxyethyl; 1-(1-methoxy-1-methyl)ethyl-carboxyloxyethyl; 1-(benzoyloxy)ethyl; isopropoxy-carboxyloxymethyl; 1-isopropoxy-carboxyloxyethyl; cyclohexyl-carboxyloxymethyl; 1-cyclohexyl-carboxyloxyethyl; cyclohexyloxy-carboxyloxymethyl; 1-cyclohexyloxy-

carbonyloxyethyl; (4-tetrahydropyranyloxy) carbonyloxymethyl; 1-(4-tetrahydropyranyloxy)carbonyloxyethyl; (4-tetrahydropyranyl)carbonyloxymethyl; and 1-(4-tetrahydropyranyl)carbonyloxyethyl).

5

Further suitable prodrug forms include phosphonate and glycolate salts. In particular, hydroxy groups (-OH), can be made into phosphonate prodrugs by reaction with chlorodibenzylphosphite, followed by hydrogenation, to form a phosphonate group -O-P(=O)(OH)₂.

10 Such a group can be cleared by phosphatase enzymes during metabolism to yield the active drug with the hydroxy group.

Also, some prodrugs are activated enzymatically to yield the active compound, or a compound which, upon further chemical reaction, yields
15 the active compound. For example, the prodrug may be a sugar derivative or other glycoside conjugate, or may be an amino acid ester derivative.

Acronyms

20 For convenience, many chemical moieties are represented using well known abbreviations, including but not limited to, methyl (Me), ethyl (Et), *n*-propyl (nPr), *iso*-propyl (iPr), *n*-butyl (nBu), *tert*-butyl (tBu), *n*-hexyl (nHex), cyclohexyl (cHex), phenyl (Ph), biphenyl (biPh), benzyl (Bn), naphthyl (naph), methoxy (MeO), ethoxy (EtO),
25 benzoyl (Bz), and acetyl (Ac).

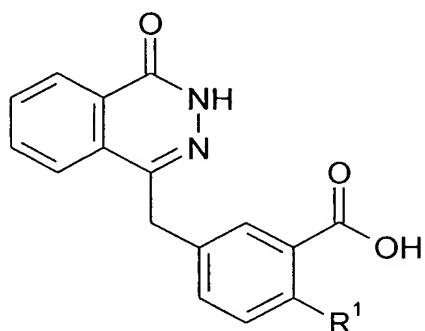
For convenience, many chemical compounds are represented using well known abbreviations, including but not limited to, methanol (MeOH), ethanol (EtOH), *iso*-propanol (i-PrOH), methyl ethyl ketone (MEK),
30 ether or diethyl ether (Et₂O), acetic acid (AcOH), dichloromethane (methylene chloride, DCM), trifluoroacetic acid (TFA), dimethylformamide (DMF), tetrahydrofuran (THF), and dimethylsulfoxide (DMSO).

35 Synthesis

In the synthesis routes given below, the A-B fused ring is shown as a fused benzene ring for convenience. Compounds in which the A-B ring is other than benzene may be synthesised using methodologies analogous to those described below by the use of appropriate alternative

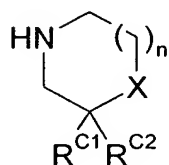
starting materials.

Compounds of the present invention may be synthesised by reaction of a compound of Formula 1:



Formula 1

in which R¹ is as previously defined, with a compound of Formula 2:

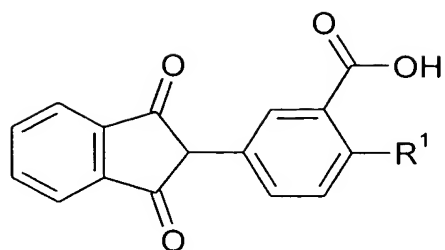


Formula 2

in which n , R^{C1} , R^{C2} and X are as previously defined, in the presence of a coupling reagent system, for example 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate or (dimethylaminopropyl)ethylcarbodiimide hydrochloride/hydroxybenzotriazole, in the presence of a base, for example diisopropylethylamine, in a solvent, for example dimethylacetamide or dichloromethane, at a temperature in the range of 0°C to the boiling point of the solvent used.

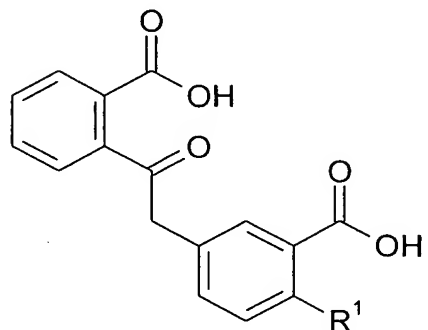
Alternatively, compounds of the present invention may be synthesised by conversion of a compound of Formula 1 into an activated species, for example an acid chloride or an activated ester such as an *N*-hydroxysuccinimide ester, using well-known methodologies, and reaction of the activated species with a compound of Formula 2.

Compounds of Formula 1 may be synthesised by reaction of a compound of Formula 3:



Formula 3

in which R¹ is as previously defined, or a compound of Formula 4:

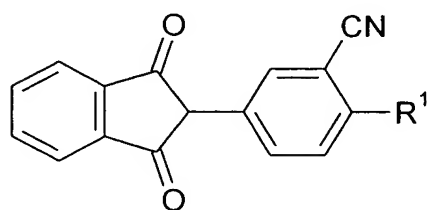


Formula 4

in which R¹ is as previously defined, or a mixture of a compound of
 5 Formula 3 and a compound of Formula 4, with a source of hydrazine, for
 example hydrazine hydrate, optionally in the presence of a base, for
 example triethylamine, optionally in the presence of a solvent, for
 example industrial methylated spirit, at a temperature in the range of
 0°C to the boiling point of the solvent used.

10

Compounds of Formula 3 or Formula 4, or mixtures thereof, may be
 synthesised by reaction of a compound of Formula 5:

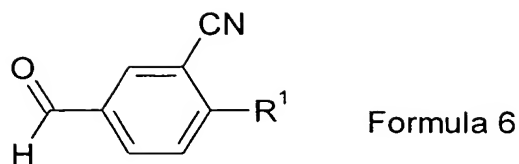


Formula 5

in which R¹ is as previously defined, with a reagent capable of
 15 hydrolysing a nitrile moiety, for example sodium hydroxide, in the
 presence of a solvent, for example water, at a temperature in the
 range of 0°C to the boiling point of the solvent used.

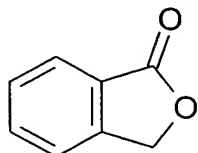
20

Compounds of Formula 5 may be synthesised by reaction of a compound of
 Formula 6:



Formula 6

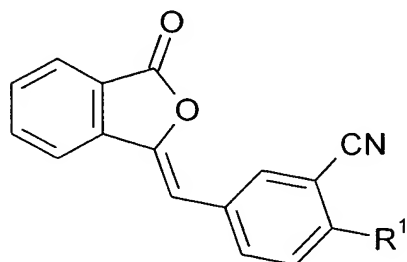
in which R¹ is as previously defined, with a compound of Formula 7:



Formula 7

in the presence of a base, for example sodium methoxide, in a solvent, for example methanol, optionally in the presence of a water scavenger, for example ethyl propionate, at a temperature in the range of 0°C to the boiling point of the solvent used.

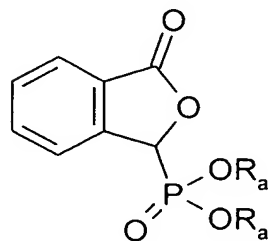
Compounds of Formula 1 may also be synthesised by reaction of a compound of Formula 8:



Formula 8

in which R¹ is as previously defined, with a reagent capable of hydrolysing a nitrile moiety, for example sodium hydroxide, in the presence of a solvent, for example water, at a temperature in the range of 0°C to the boiling point of the solvent used, followed by reaction of the resulting intermediate with a source of hydrazine, for example hydrazine hydrate, at a temperature in the range of 0°C to the boiling point of the solvent used.

Compounds of Formula 8 may be synthesised by reaction of a compound of Formula 9:



Formula 9

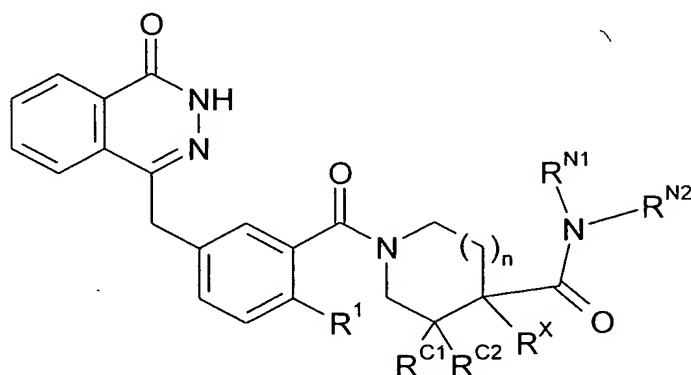
in which R_a is a C_{1-4} alkyl group, with a compound of Formula 6, in the presence of a base, for example triethylamine or lithium hexamethyldisilazide, in the presence of a solvent, for example tetrahydrofuran, at a temperature in the range of -80°C to the boiling point of the solvent used.

Compounds of Formula 9 may be synthesised by methods analogous to those described in WO 02/26576.

- 10 Compounds of Formula 1 may also be synthesised by methods analogous to those described above in which the nitrile moiety in all Formulae is replaced by other moieties capable of generating a carboxylic acid, for example ester or carboxamide moieties.
- 15 Compounds of Formula 2 are commercially available or may be synthesised by methods reported in the chemical literature.

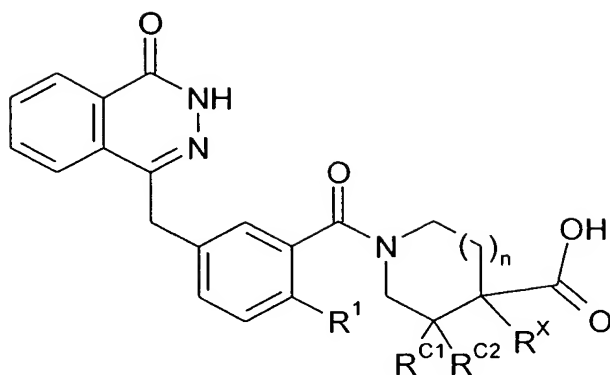
Compounds of the present invention in which X is CR^XR^Y , in which one of R^X or R^Y is an amido moiety, and which may therefore be represented by

20 Formula 10:



Formula 10

- in which n , $\text{R}^{\text{C}1}$, $\text{R}^{\text{C}2}$, R^1 and R^X are as previously defined and $\text{R}^{\text{N}1}$ and $\text{R}^{\text{N}2}$ are each individually selected from the group consisting of H, optionally substituted C_{1-20} alkyl, C_{5-20} aryl, C_{3-20} heterocyclyl, or may together form an optionally substituted C_{3-7} cycloalkyl or heterocyclyl group, may be synthesised by reaction of a compound of Formula 11:

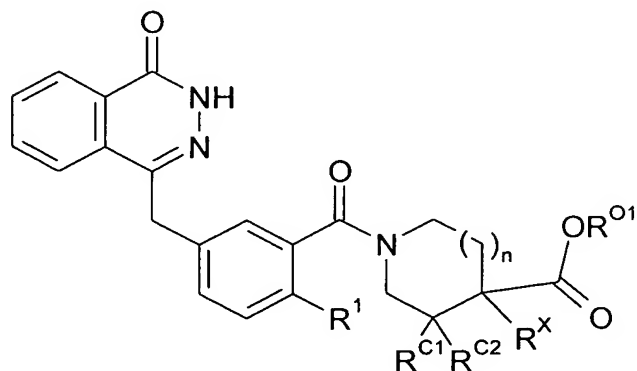


Formula 11

in which n , R^{C1} , R^{C2} , R^1 and R^X are as previously defined, with a compound of Formula $HNR^{N1}R^{N2}$, in which R^{N1} and R^{N2} are as previously defined, in the presence of a coupling reagent system, for example 2-
 5 (1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate or (dimethylaminopropyl)ethylcarbodiimide hydrochloride/ hydroxybenzotriazole, in the presence of a base, for example diisopropylethylamine, in a solvent, for example
 10 dimethylacetamide or dichloromethane, at a temperature in the range of 0°C to the boiling point of the solvent used.

Alternatively, compounds of Formula 10 may be synthesised by conversion of a compound of Formula 11 into an activated species, for
 15 example an acid chloride or an activated ester such as an *N*-hydroxysuccinimide ester, using well-known methodologies, and reaction of the activated species with a compound of Formula $HNR^{N1}R^{N2}$.

Compounds of Formula 11 may be synthesised by deprotection of a
 20 protected form of a compound of Formula 11, for example a compound of Formula 12:



Formula 12

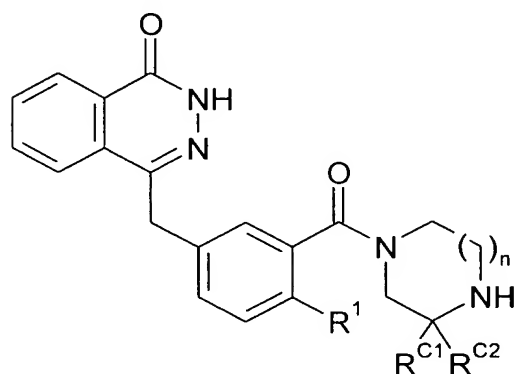
in which n , R^{C1} , R^{C2} , R^1 and R^X are as previously defined and R^{O1} is a C_1 -

4 alkyl group, using well known methodologies, for example base-catalysed hydrolysis in the presence of a source of hydroxide, for example sodium or lithium hydroxide, in the presence of a solvent, for example water and/or tetrahydrofuran, at a temperature in the range of
 5 0°C to the boiling point of the solvent used.

Compounds of Formula 12 may be synthesised from compounds of Formula 1 by the previously described methods.

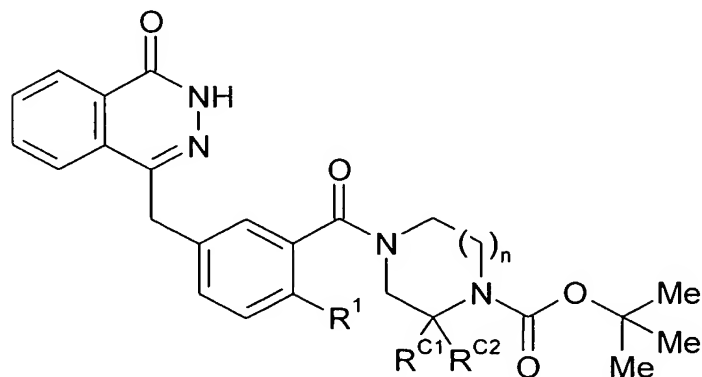
10 Compounds of Formula $\text{HNR}^{\text{N1}}\text{R}^{\text{N2}}$ are commercially available or may be synthesised by methods reported in the chemical literature.

Compounds of the present invention in which X is NH and which may therefore be represented by Formula 13:



Formula 13

15 in which n , R^{C1} , R^{C2} and R^1 are as previously defined, may be synthesised by deprotection of a protected form of a compound of Formula 13, for example a compound of Formula 14:



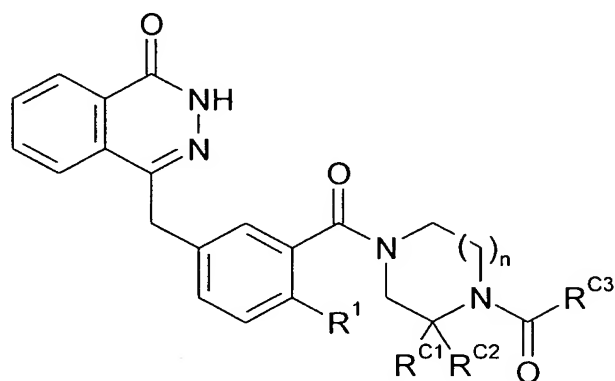
Formula 14

20 in which n , R^{C1} , R^{C2} and R^1 are as previously defined, using well known methodologies, for example acid-catalysed cleavage, in the presence of an acid, for example trifluoroacetic acid or hydrochloric acid, in the presence of a solvent, for example dichloromethane or ethanol and/or

water, at a temperature in the range of 0°C to the boiling point of the solvent used.

Compounds of Formula 14 may be synthesised from compounds of Formula 1
5 by the previously described methods.

Compounds of the present invention in which X is NR^x, in which R^x is an acyl moiety, and which may therefore be represented by Formula 15:



Formula 15

10 in which n, R^{C1}, R^{C2} and R¹ are as previously defined and R^{C3} is selected from the group consisting of optionally substituted C₁₋₂₀ alkyl, C₅₋₂₀ aryl and C₃₋₂₀ heterocyclyl, may be synthesised by reaction of a compound of Formula 13 with a compound of Formula R^{C3}COX, in which R^{C3}
15 is as previously defined and X is a suitable leaving group, for example a halogen such as chloro, optionally in the presence of a base, for example pyridine, triethylamine or diisopropylethylamine, optionally in the presence of a solvent, for example dichloromethane, at a temperature in the range of 0°C to the boiling point of the solvent used.

20 Compounds of Formula R^{C3}COX are commercially available or may be synthesised by methods reported in the chemical literature.

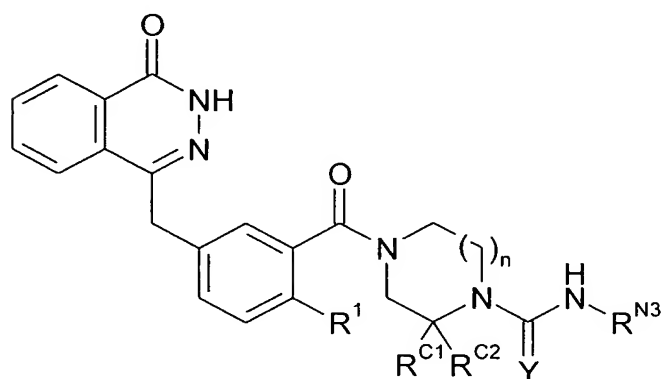
Compounds of Formula 15 may also be synthesised by reaction of a
25 compound of Formula 13 with a compound of Formula R^{C3}CO₂H, in which R^{C3} is as previously defined, in the presence of a coupling reagent system, for example 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate or
30 (dimethylaminopropyl)ethylcarbodiimide hydrochloride/ hydroxybenzotriazole, in the presence of a base, for example

diisopropylethylamine, in a solvent, for example dimethylacetamide or dichloromethane, at a temperature in the range of 0°C to the boiling point of the solvent used.

- 5 Compounds of Formula $R^{C3}CO_2H$ are commercially available or may be synthesised by methods reported in the chemical literature.

Compounds of the present invention in which X is NR^x , in which R^x is an amido or thioamido moiety, and which may therefore be represented by

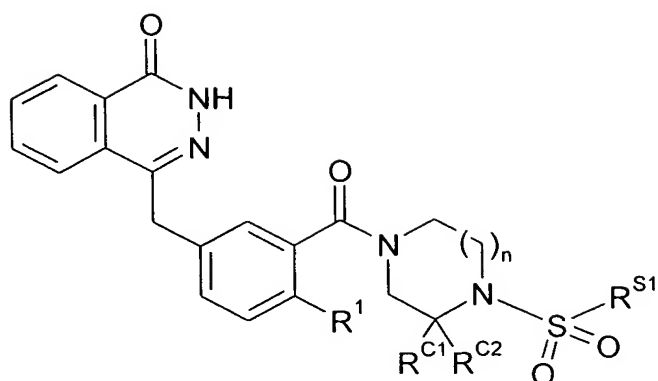
10 Formula 16:



Formula 16

- in which n , R^{C1} , R^{C2} and R^1 are as previously defined, Y is O or S and R^{N3} is selected from the group consisting of optionally substituted C_{1-20} alkyl, C_{5-20} aryl and C_{3-20} heterocyclyl, may be synthesised by reaction
- 15 of a compound of Formula 13 with a compound of Formula $R^{N3}NCY$, in which Y and R^{N3} are as previously defined, in the presence of a solvent, for example dichloromethane, at a temperature in the range of 0°C to the boiling point of the solvent used.
- 20 Compounds of Formula $R^{N3}NCY$ are commercially available or may be synthesised by methods reported in the chemical literature.

Compounds of the present invention in which X is NR^x , in which R^x is a sulfonyl moiety, and which may therefore be represented by Formula 17:

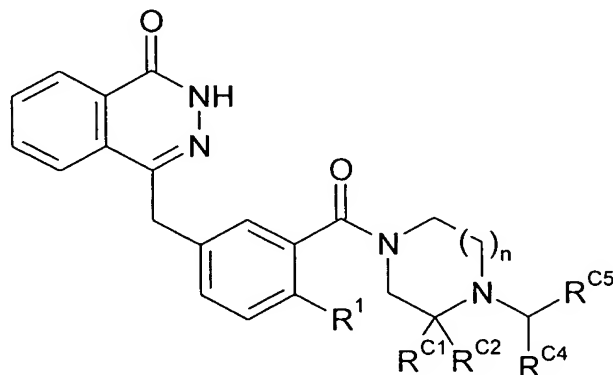


Formula 17

in which n , R^{C1} , R^{C2} and R^1 are as previously defined and R^{S1} is selected from the group consisting of optionally substituted C_{1-20} alkyl, C_{5-20} aryl and C_{3-20} heterocyclyl, may be synthesised by reaction of a compound of Formula 13 with a compound of Formula $R^{S1}SO_2Cl$, in which R^{S1} is as previously defined, optionally in the presence of a base, for example pyridine, triethylamine or diisopropylethylamine, in the presence of a solvent, for example dichloromethane, at a temperature in the range of $0^\circ C$ to the boiling point of the solvent used.

Compounds of Formula $R^{S1}SO_2Cl$ are commercially available or may be synthesised by methods reported in the chemical literature.

Compounds of the present invention in which X is NR^X , in which R^X is selected from the group consisting of optionally substituted C_{1-20} alkyl or C_{3-20} heterocyclyl, and which may therefore be represented by Formula 18:



Formula 18

in which n , R^{C1} , R^{C2} and R^1 are as previously defined and R^{C4} and R^{C5} are each individually selected from the group consisting of H, optionally substituted C_{1-20} alkyl, C_{5-20} aryl, C_{3-20} heterocyclyl, or may together form an optionally substituted C_{3-7} cycloalkyl or heterocyclyl group, may be synthesised by reaction of a compound of Formula 13 with a

compound of Formula $R^{C4}COR^{C5}$, in which R^{C4} and R^{C5} are as previously defined, in the presence of a reducing agent, for example sodium cyanoborohydride or sodium triacetoxyborohydride, in the presence of a solvent, for example methanol, optionally in the presence of an acid catalyst, for example acetic acid, at a temperature in the range of 0°C to the boiling point of the solvent used.

Compounds of Formula $R^{C4}COR^{C5}$ are commercially available or may be synthesised by methods reported in the chemical literature.

Use

The present invention provides active compounds, specifically, active in inhibiting the activity of PARP.

The term "active" as used herein, pertains to compounds which are capable of inhibiting PARP activity, and specifically includes both compounds with intrinsic activity (drugs) as well as prodrugs of such compounds, which prodrugs may themselves exhibit little or no intrinsic activity.

One assay which may conveniently be used in order to assess the PARP inhibition offered by a particular compound is described in the examples below.

The present invention further provides a method of inhibiting the activity of PARP in a cell, comprising contacting said cell with an effective amount of an active compound, preferably in the form of a pharmaceutically acceptable composition. Such a method may be practised *in vitro* or *in vivo*.

For example, a sample of cells may be grown *in vitro* and an active compound brought into contact with said cells, and the effect of the compound on those cells observed. As examples of "effect", the amount of DNA repair effected in a certain time may be determined. Where the active compound is found to exert an influence on the cells, this may be used as a prognostic or diagnostic marker of the efficacy of the compound in methods of treating a patient carrying cells of the same cellular type.

The term "treatment", as used herein in the context of treating a condition, pertains generally to treatment and therapy, whether of a human or an animal (e.g. in veterinary applications), in which some desired therapeutic effect is achieved, for example, the inhibition of the progress of the condition, and includes a reduction in the rate of progress, a halt in the rate of progress, amelioration of the condition, and cure of the condition. Treatment as a prophylactic measure (i.e. prophylaxis) is also included.

- 10 The term "adjunct" as used herein relates to the use of active compounds in conjunction with known therapeutic means. Such means include cytotoxic regimes of drugs and/or ionising radiation as used in the treatment of different cancer types. In particular, the active compounds are known to potentiate the actions of a number of cancer
- 15 chemotherapy treatments, which include the topoisomerase class of poisons (e.g. topotecan, irinotecan, rubitecan), most of the known alkylating agents (e.g. DTIC, temozolamide) and platinum based drugs (e.g. carboplatin, cisplatin) used in treating cancer.
- 20 Active compounds may also be used as cell culture additives to inhibit PARP, for example, in order to sensitize cells to known chemotherapeutic agents or ionising radiation treatments *in vitro*.

Active compounds may also be used as part of an *in vitro* assay, for example, in order to determine whether a candidate host is likely to benefit from treatment with the compound in question.

Administration

- The active compound or pharmaceutical composition comprising the
- 30 active compound may be administered to a subject by any convenient route of administration, whether systemically/ peripherally or at the site of desired action, including but not limited to, oral (e.g. by ingestion); topical (including e.g. transdermal, intranasal, ocular, buccal, and sublingual); pulmonary (e.g. by inhalation or insufflation
- 35 therapy using, e.g. an aerosol, e.g. through mouth or nose); rectal; vaginal; parenteral, for example, by injection, including subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intracardiac, intrathecal, intraspinal, intracapsular, subcapsular, intraorbital, intraperitoneal, intratracheal, subcuticular,

intraarticular, subarachnoid, and intrasternal; by implant of a depot, for example, subcutaneously or intramuscularly.

The subject may be a eukaryote, an animal, a vertebrate animal, a mammal, a rodent (e.g. a guinea pig, a hamster, a rat, a mouse), murine (e.g. a mouse), canine (e.g. a dog), feline (e.g. a cat), equine (e.g. a horse), a primate, simian (e.g. a monkey or ape), a monkey (e.g. marmoset, baboon), an ape (e.g. gorilla, chimpanzee, orangutang, gibbon), or a human.

Formulations

While it is possible for the active compound to be administered alone, it is preferable to present it as a pharmaceutical composition (e.g., formulation) comprising at least one active compound, as defined above, together with one or more pharmaceutically acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, preservatives, lubricants, or other materials well known to those skilled in the art and optionally other therapeutic or prophylactic agents.

Thus, the present invention further provides pharmaceutical compositions, as defined above, and methods of making a pharmaceutical composition comprising admixing at least one active compound, as defined above, together with one or more pharmaceutically acceptable carriers, excipients, buffers, adjuvants, stabilisers, or other materials, as described herein.

The term "pharmaceutically acceptable" as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g. human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be "acceptable" in the sense of being compatible with the other ingredients of the formulation.

Suitable carriers, diluents, excipients, etc. can be found in standard pharmaceutical texts. See, for example, "Handbook of Pharmaceutical Additives", 2nd Edition (eds. M. Ash and I. Ash), 2001 (Synapse

Information Resources, Inc., Endicott, New York, USA), "Remington's Pharmaceutical Sciences", 20th edition, pub. Lippincott, Williams & Wilkins, 2000; and "Handbook of Pharmaceutical Excipients", 2nd edition, 1994.

5

The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active compound with the carrier which constitutes one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the active compound with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

10

Formulations may be in the form of liquids, solutions, suspensions, emulsions, elixirs, syrups, tablets, lozenges, granules, powders, capsules, cachets, pills, ampoules, suppositories, pessaries, ointments, gels, pastes, creams, sprays, mists, foams, lotions, oils, boluses, electuaries, or aerosols.

20

Formulations suitable for oral administration (e.g., by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

25

A tablet may be made by conventional means, e.g. compression or molding, optionally with one or more accessory ingredients.

Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules, optionally mixed with one or more binders (e.g. povidone, gelatin, acacia, sorbitol, tragacanth, hydroxypropylmethyl cellulose); fillers or diluents (e.g. lactose, microcrystalline cellulose, calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc, silica); disintegrants (e.g. sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose); surface-active or dispersing or wetting agents (e.g., sodium lauryl sulfate); and preservatives (e.g., methyl p-hydroxybenzoate, propyl p-

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hydroxybenzoate, sorbic acid). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active compound therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Formulations suitable for topical administration (e.g. transdermal, intranasal, ocular, buccal, and sublingual) may be formulated as an ointment, cream, suspension, lotion, powder, solution, past, gel, spray, aerosol, or oil. Alternatively, a formulation may comprise a patch or a dressing such as a bandage or adhesive plaster impregnated with active compounds and optionally one or more excipients or diluents.

Formulations suitable for topical administration in the mouth include lozenges comprising the active compound in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active compound in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active compound in a suitable liquid carrier.

Formulations suitable for topical administration to the eye also include eye drops wherein the active compound is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active compound.

Formulations suitable for nasal administration, wherein the carrier is a solid, include a coarse powder having a particle size, for example, in the range of about 20 to about 500 microns which is administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nasal passage from a container of the powder held close up to the nose. Suitable formulations wherein the carrier is a liquid for administration as, for example, nasal spray, nasal drops, or by aerosol administration by nebuliser, include aqueous or oily solutions of the active compound.

Formulations suitable for administration by inhalation include those presented as an aerosol spray from a pressurised pack, with the use of a suitable propellant, such as dichlorodifluoromethane,
5 trichlorofluoromethane, dichloro-tetrafluoroethane, carbon dioxide, or other suitable gases.

Formulations suitable for topical administration via the skin include ointments, creams, and emulsions. When formulated in an ointment, the
10 active compound may optionally be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active compounds may be formulated in a cream with an oil-in-water cream base. If desired, the aqueous phase of the cream base may include, for example, at least about 30% w/w of a polyhydric alcohol, i.e., an alcohol
15 having two or more hydroxyl groups such as propylene glycol, butane-1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active compound through the skin or other affected areas. Examples of such
20 dermal penetration enhancers include dimethylsulfoxide and related analogues.

When formulated as a topical emulsion, the oily phase may optionally comprise merely an emulsifier (otherwise known as an emulgent), or it
25 may comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabiliser. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabiliser(s) make
30 up the so-called emulsifying wax, and the wax together with the oil and/or fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Suitable emulgents and emulsion stabilisers include Tween 60, Span 80,
35 cetostearyl alcohol, myristyl alcohol, glyceryl monostearate and sodium lauryl sulphate. The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties, since the solubility of the active compound in most oils likely to be used in pharmaceutical emulsion formulations may be very low. Thus

the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active compound, such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration (e.g., by injection, including cutaneous, subcutaneous, intramuscular, intravenous and intradermal), include aqueous and non-aqueous isotonic, pyrogen-free, sterile injection solutions which may contain anti-oxidants, buffers, preservatives, stabilisers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. Examples of suitable isotonic vehicles for use in such formulations include Sodium Chloride Injection, Ringer=s Solution, or Lactated Ringer=s Injection. Typically, the concentration of the active compound in the solution is from about 1 ng/ml to about 10 µg/ml, for example from about 10 ng/ml to about 1 µg/ml. The formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier,

for example water for injections, immediately prior to use.

Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, and tablets. Formulations may be in the form of liposomes or other microparticulate systems which are designed to target the active compound to blood components or one or more organs.

Dosage

It will be appreciated that appropriate dosages of the active compounds, and compositions comprising the active compounds, can vary from patient to patient. Determining the optimal dosage will generally involve the balancing of the level of therapeutic benefit against any risk or deleterious side effects of the treatments of the present invention. The selected dosage level will depend on a variety of factors including, but not limited to, the activity of the particular compound, the route of administration, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds, and/or materials used in combination, and the age, sex, weight, condition, general health, and prior medical history of the patient. The amount of compound and route of administration will ultimately be at the discretion of the physician, although generally the dosage will be to achieve local concentrations at the site of action which achieve the desired effect without causing substantial harmful or deleterious side-effects.

Administration *in vivo* can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the formulation used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

In general, a suitable dose of the active compound is in the range of about 100 μg to about 250 mg per kilogram body weight of the subject per day. Where the active compound is a salt, an ester, prodrug, or the like, the amount administered is calculated on the basis of the

parent compound and so the actual weight to be used is increased proportionately.

Synthesis Data

5 General Experimental Methods

Preparative HPLC

Samples were purified with a Waters mass-directed purification system utilising a Waters 600 LC pump, Waters Xterra C18 column (5 μ m 19 mm x 50 mm) and Micromass ZQ mass spectrometer, operating in positive ion electrospray ionisation mode. Mobile phases A (0.1% formic acid in water) and B (0.1 % formic acid in acetonitrile) were used in a gradient; 5% B to 100% over 7 min, held for 3 min, at a flow rate of 20 ml/ min.

15 Analytical HPLC-MS

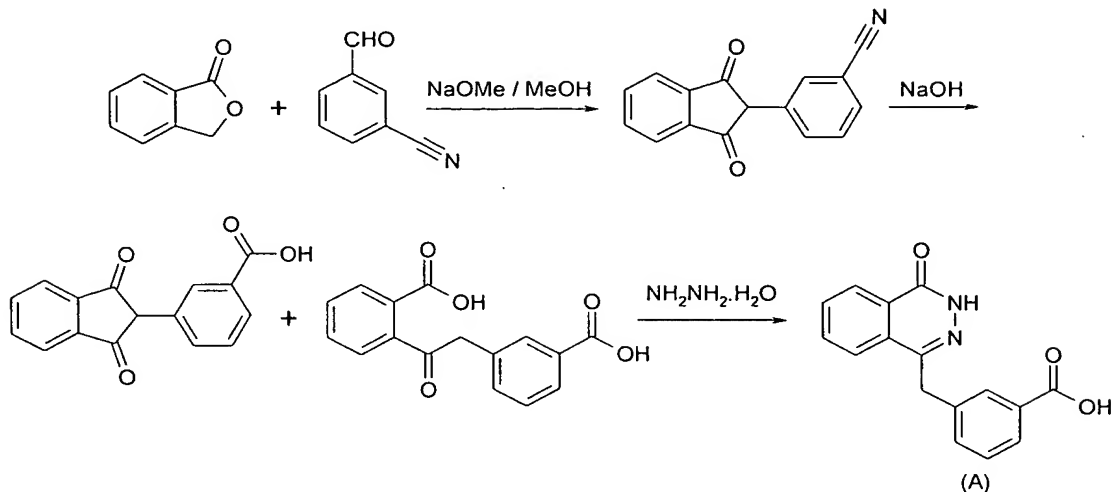
Analytical HPLC was carried out with a Spectra System P4000 pump and Jones Genesis C18 column (4 μ m, 50 mm x 4.6 mm). Mobile phases A (0.1 % formic acid in water) and B (acetonitrile) were used in a gradient of 5 % B for 1 min rising to 98 % B after 5 min, held for 3 min at a flow rate of 2 ml / min. Detection was by a TSP UV 6000LP detector at 254 nm UV and range 210-600 nm PDA. The Mass spectrometer was a Finnigan LCQ operating in positive ion electrospray mode.

NMR

25 ^1H NMR and ^{13}C NMR were recorded using Bruker DPX 300 spectrometer at 300 MHz and 75 MHz respectively. Chemical shifts were reported in parts per million (ppm) on the δ scale relative to tetramethylsilane internal standard. Unless stated otherwise all samples were dissolved in DMSO- d_6 .

Synthesis of Key Intermediates

a. 3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A)



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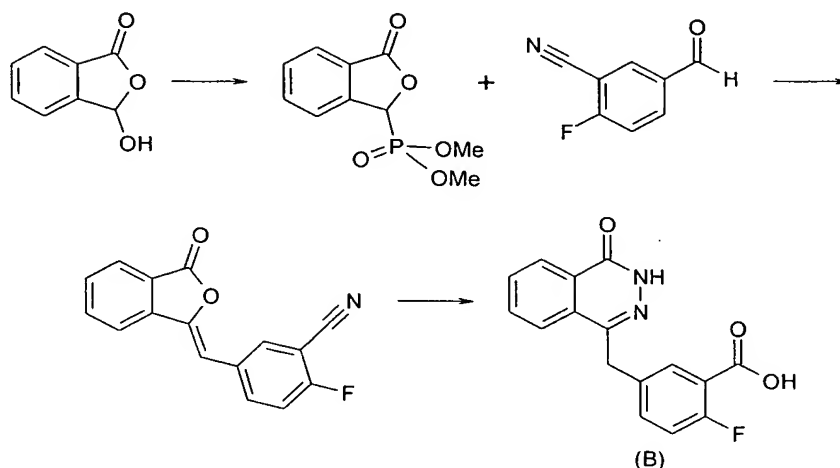
A mixture of 27% sodium methoxide solution in methanol (400 g, 2 mol) and methanol (150 ml) was added dropwise between ambient temperature and 30°C over 15 minutes to a stirred mixture of phthalide (67 g, 0.5 mol), 3-formylbenzonitrile (65.5 g, 0.5 mol) and ethyl propionate (250 ml), the mixture was stirred at ambient temperature for 40 minutes and at reflux temperature for 1 hour, then it was allowed to cool to ambient temperature. The resulting red solid was collected by filtration, washed with ethyl acetate (2 x 50 ml) and dissolved in water (1800 ml). The solution was acidified by the addition of acetic acid (60 ml) and the resulting red solid was collected by filtration, washed with water (2 x 200 ml) and dried *in vacuo* to give 3-(1,3-dioxoindan-2-yl)benzonitrile (83.2 g) as a dark red solid, m.pt. 179-182°C, m/z (M+H)⁺ 248, which was used without further purification.

3-(1,3-Dioxoindan-2-yl)benzonitrile (74.18 g, 0.3 mol) was added in portions to a solution of sodium hydroxide (36 g, 0.9 mol) in water (580 ml), the resulting dark red suspension was stirred at reflux temperature for 5 hours, then it was cooled to ambient temperature and washed with ethyl acetate (3 x 300 ml). The aqueous solution was acidified by the dropwise addition of concentrated hydrochloric acid (110 ml), the mixture was stirred at ambient temperature for 1 hour, then the resulting solid was collected by filtration, washed with water (2 x 200 ml) and dried *in vacuo* to give a 1:1 mixture of 3-(1,3-

dioxoindan-2-yl)benzoic acid, $(M+H)^+$ 267, and 2-[2-(3-carboxyphenyl)acetyl]benzoic acid, $(M+H)^+$ 285, (69.32 g), which was used without further purification.

5 The mixture obtained in the previous step (52.8 g) was added to a solution of triethylamine (37.55 g, 0.372 mol) in industrial methylated spirit (500 ml) and the resulting cloudy solution was filtered through a pad of filter-aid to give a clear solution. Hydrazine monohydrate (9.3 g, 0.186 mol) was added in one portion at
10 ambient temperature, the stirred mixture was heated under reflux for 1 hour, then it was concentrated *in vacuo* to approximately 250 ml and added to a solution of sodium acetate (41 g, 0.5 mol) in water (500 ml). The mixture was brought to pH 7 by the dropwise addition of concentrated hydrochloric acid, then it was stirred at ambient
15 temperature for 3 hours. The resulting solid was collected by filtration, washed with water (50 ml) and dried *in vacuo* to give a white solid (15.62 g). The combined filtrate and washings were acidified to pH 6 by the addition of hydrochloric acid, then the mixture was stirred at ambient temperature for 3 hours. The resulting
20 solid was collected by filtration, washed with water (50 ml) and dried *in vacuo* to give a second crop of off-white solid (17.57 g). The combined filtrate and washings from the second crop were readjusted to pH 6 and treated as before to give a third crop of pale orange solid (6.66 g). The three crops were combined to give essentially pure 3-
25 (4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A), $(M+H)^+$ 281, δ_H 4.4 (2H, s), 7.2-7.4 (1H, m), 7.5-7.6 (1H, m), 7.7-8.0 (5H, m), 8.1-8.2 (1H, m), 12.6 (1H, s)

b. 2-Fluoro-5-(4-oxo-3,4-dihydro-phthalazin-1-ylmethyl)benzoic acid (B).

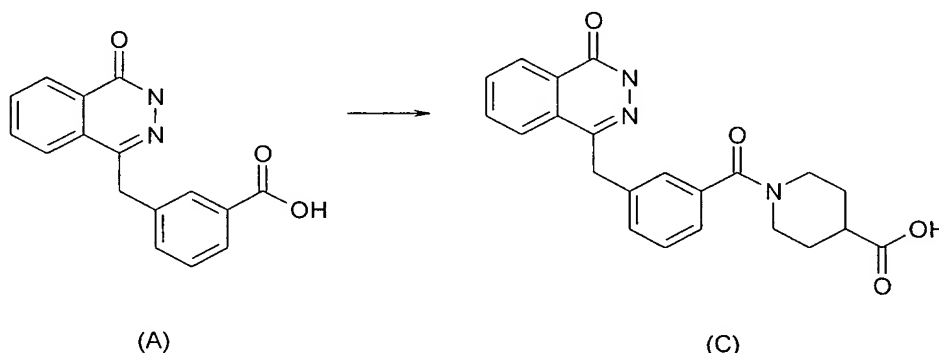


5 Dimethyl phosphite (22.0 g, 0.2 mol) was added drop-wise to a solution of sodium methoxide (43.0 g) in methanol (100 ml) at 0°C. 2-Carboxybenzaldehyde (21.0 g, 0.1 mol) was then added portion-wise to the reaction mixture as a slurry in methanol (40 ml), with the temperature kept below 5°C. The resulting pale yellow solution was
 10 warmed to 20°C over 1 hour. Methanesulphonic acid (21.2 g, 0.22 mol) was added to the reaction drop-wise and the resulting white suspension was evaporated *in vacuo*. The white residue was quenched with water and extracted into chloroform (3 x 100 ml). The combined organic extracts were washed with water (2 x 100 ml), dried over MgSO₄, and evaporated
 15 *in vacuo* to yield (3-oxo-1,3-dihydro-isobenzofuran-1-yl)phosphonic acid dimethyl ester as a white solid (32.0 g, 95 %, 95 % purity). This was then used without further purification in the next stage.

To a mixture of (3-oxo-1,3-dihydro-isobenzofuran-1-yl)phosphonic acid
 20 dimethyl ester (35.0 g, 0.14 mol) in tetrahydrofuran (200 ml) and 2-fluoro-5-formylbenzonitrile (20.9 g, 0.14 mol) in tetrahydrofuran (130 ml) was added triethylamine (14 ml, 0.14 mol) drop-wise over 25 min, with the temperature kept below 15°C. The reaction mixture was warmed slowly to 20°C over 1 hour and concentrated *in vacuo*. The white
 25 residue was slurried in water (250 ml) for 30 minutes, filtered, washed with water, hexane and ether, and dried to yield 2-fluoro-5-(3-oxo-3H-isobenzofuran-1-ylidenemethyl)benzonitrile as a 50:50 mixture of E and Z isomers (37.2 g, 96 %);
 m/z [M+1]⁺ 266 (98 % purity)

To a suspension of 2-fluoro-5-(3-oxo-3H-isobenzofuran-1-ylidenemethyl)benzonitrile in water (200 ml) was added aqueous sodium hydroxide (26.1 g in 50 ml water) solution and the reaction mixture was heated under nitrogen to 90°C for 30 minutes. The reaction mixture was partially cooled to 70°C, and hydrazine hydrate (100 ml) was added and stirred for 18 hours at 70°C. The reaction was cooled to room temperature and acidified with 2M HCl to pH 4. The mixture was stirred for 10 min and filtered. The resulting solid was washed with water, hexane, ether, ethyl acetate and dried to yield 2-fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid as a pale pink powder (30.0 g, 77 %). m/z $[M+1]^+$ 299 (96 % purity), δ_H 4.4 (2H, s), 7.2-7.3 (1H, m), 7.5-7.6 (1H, m), 7.8-8.0 (4H, m), 8.2-8.3 (1H, m), 12.6 (1H, s).

c. 1-[3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-4-carboxylic acid (C)

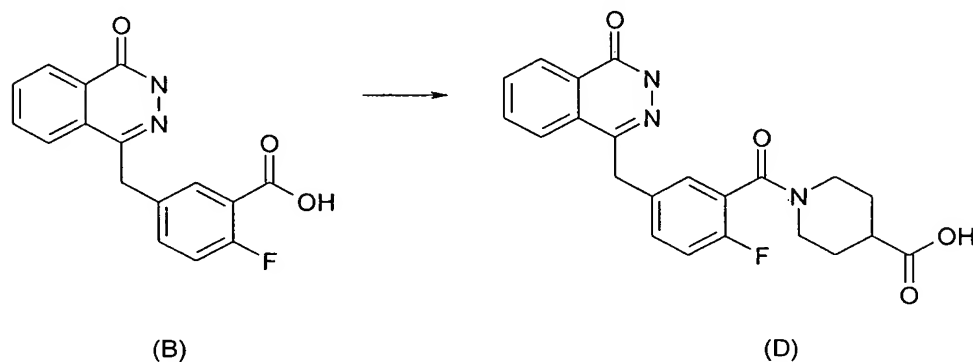


3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A) (7.0 g, 0.25 mol), ethyl isonipecotate (5 ml, 0.32 mol), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (12.3 g, 0.32 mol) and N,N-diisopropylethylamine (10.0 ml, 0.55 mol) were added to dimethylacetamide (40 ml) and stirred for 18 h. Water (100 ml) was added to the reaction mixture and the product was extracted into dichloromethane (4 x 50 ml). The combined organic layers were washed with water (3 x 100 ml), dried over $MgSO_4$, filtered and evaporated *in vacuo* to yield an oil. To a solution of the oil in tetrahydrofuran (100 ml) was added 10 % aqueous sodium hydroxide solution (20 ml) and the reaction was stirred for 18 hours. The reaction was concentrated, washed with ethyl acetate (2 x 30 ml) and acidified with 2M HCl to pH 2. The aqueous layer was extracted with dichloromethane (2 x 100 ml), then the extracts were dried over $MgSO_4$, filtered and evaporated to yield 1-[3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-

4-carboxylic acid (C) as a yellow solid (7.0 g, 65 %), m/z $[M+1]^+$ 392 (96 % purity), δ_H 1.3-1.8 (5H, m), 2.8-3.1 (4H, m), 4.4 (2H, s), 7.2-7.3 (1H, m), 7.3-7.4 (1H, m), 7.7-8.0 (5H, m), 8.2-8.3 (1H, m), 12.6 (1H, s).

5

d. 1-[2-Fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-4-carboxylic acid (D)



2-Fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid

10 (B) (3.1 g, 0.14 mol), ethyl isonipecotat (1.7 ml, 0.11 mol), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (5.1 g, 0.13 mol) and N,N-diisopropylethylamine (10.0 ml, 0.55 mol) were added to dimethylacetamide (15 ml) and stirred for 18 hours. Water (100 ml) was added to the reaction mixture and the product was

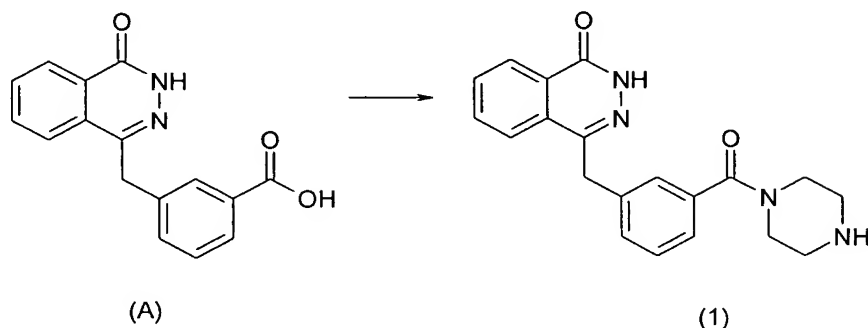
15 extracted into dichloromethane (4 x 50 ml). The combined organic layers were, filtered, washed with water (3 x 100 ml), dried over $MgSO_4$, filtered and evaporated *in vacuo* to yield an orange oil. The oil was purified by flash chromatography (ethyl acetate) to yield 1-[2-fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-4-

20 carboxylic acid as the methyl ester (1.5 g, 33 %, 96 % purity). To a solution of the methyl ester in tetrahydrofuran: water (2:1, 40 ml) was added sodium hydroxide (0.3 g, 0.075 mol) and the reaction was stirred for 18 h. The reaction was concentrated, washed with ethyl acetate (2 x 20 ml) and acidified with 2M HCl to pH 2. The aqueous

25 layer was extracted with dichloromethane (2 x 20 ml), and the combined extracts were dried over $MgSO_4$ and evaporated to yield 1-[3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]piperidine-4-carboxylic acid (D) as a yellow solid (0.6 g, 65 %), m/z $[M+1]^+$ 392 (96 % purity)

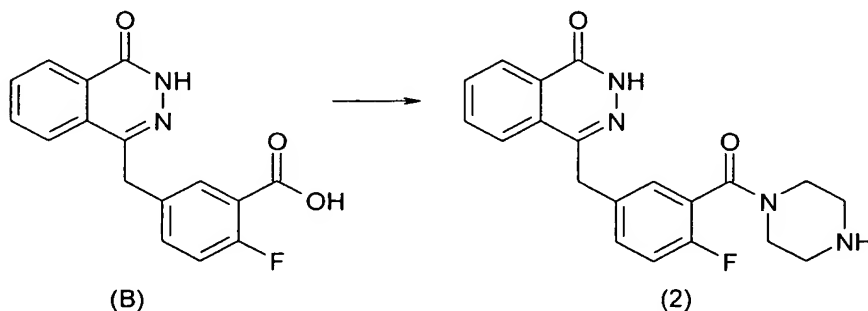
Example 1 - Synthesis of Key Compounds

a. Synthesis of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1)



3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A) (5.0g, 0.17mol), *tert*-butyl 1-piperazinecarboxylate (3.9 g, 0.21 mol), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (8.6 g, 0.22 mol) and *N,N*,*N*,*N*-diisopropylethylamine (6.7 ml, 0.38 mol) were added to dimethylacetamide (40 ml) and stirred for 18 hours. Water (100 ml) was added and the reaction mixture was heated to 100°C for 1 hour. The suspension was cooled to room temperature, filtered and dried to yield a white solid. The solid was dissolved in a solution of 6M HCl and ethanol (2:1, 50 ml) and stirred for 1 hour. The reaction was concentrated, basified with ammonia to pH 9, and the product was extracted into dichloromethane (2 x 50 ml). The combined organic layers were washed with water (2 x 50 ml), dried over MgSO₄, and evaporated *in vacuo* to yield 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) as a yellow crystalline solid (4.0 g, 77 %); *m/z* [M+1]⁺ 349 (97 % purity), δ_H 2.6-3.8 (8H, m), 4.4 (2H, s), 7.2-7.5 (4H, m), 7.7-8.0 (3H, m), 8.2-8.3 (1H, m), 12.6 (1H, s)

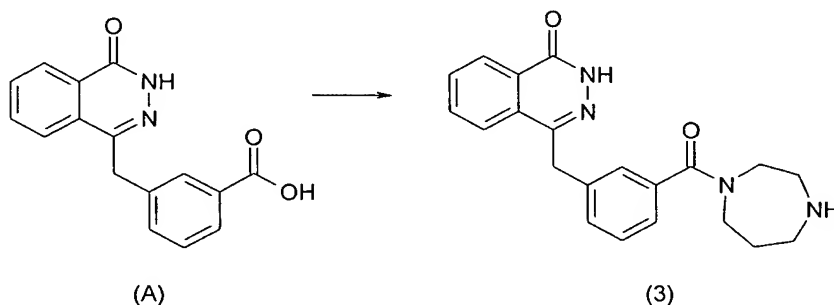
b. Synthesis of 4-[4-Fluoro-3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (2)



The synthesis was carried out according to the method described in (a)

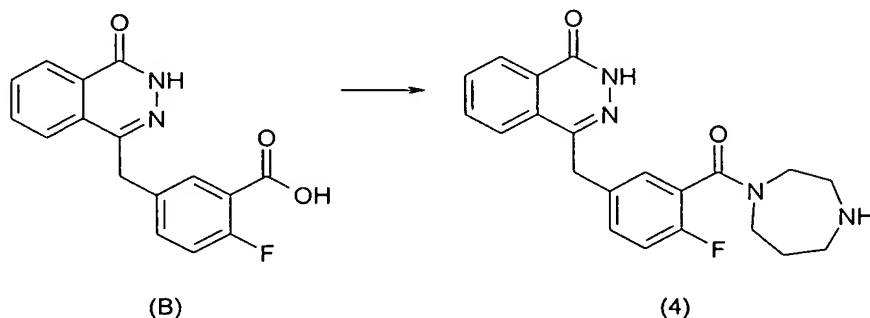
above using 2-fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (B) to yield 4-[4-fluoro-3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (2) as a white crystalline solid (4.8 g, 76 %); m/z $[M+1]^+$ 367 (97 % purity), δ_H 2.6-3.8 (8H, m), 4.4 (2H, s), 7.2-7.5 (3H, m), 7.7-8.0 (3H, m), 8.2-8.3 (1H, m), 12.6 (1H, s).

c. Synthesis of 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3)



10 The synthesis was carried out according to the method described in (a) above using 3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A) and *tert*-butyl 1-homopiperazine carboxylate to yield 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3) as a grey crystalline solid (5.3 g, 97 %); m/z $[M+1]^+$ 363 (97 % purity); δ_H 2.6-3.8 (10H, m), 4.4 (2H, s), 7.2-7.5 (4H, m), 7.7-8.0 (3H, m), 8.2-8.3 (1H, m), 12.6 (1H, s).

d. Synthesis of 4-[3-([1,4]diazepane-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one (4)

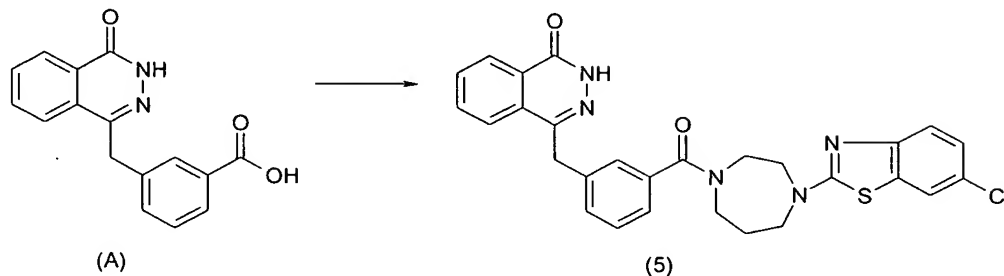


20 The synthesis was carried out according to the method described in (a) above using 2-fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (B) and *tert*-butyl 1-homopiperazinecarboxylate to yield 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (4) as a yellow crystalline solid (5.3 g, 68 %); m/z $[M+1]^+$ 381 (97 % purity); δ_H 2.6-3.8 (10H, m), 4.4 (2H, s), 7.2-7.5 (3H, m), 7.7-8.0 (3H, m), 8.2-8.3

(1H, m), 12.6 (1H, s).

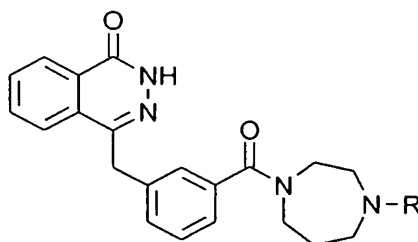
Example 2

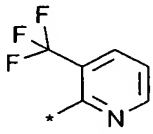
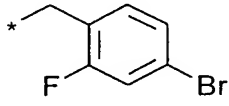
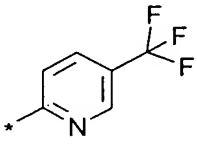
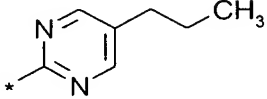
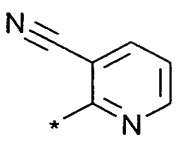
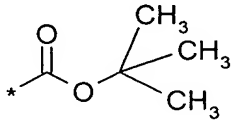
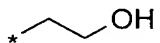
a. 4-{3-[4-(6-Chlorobenzothiazol-2-yl)-1,4-diazepan-1-ylcarbonyl]benzyl}-1(2H)-phthalazinone

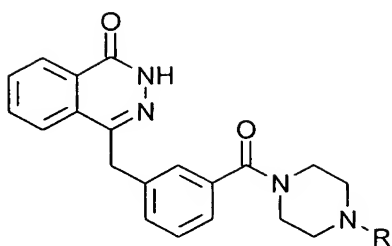


2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (150 mg, 0.47 mmol), diisopropylethylamine (102 mg, 0.8 mmol) and 6-chloro-2-(1,4-diazepan-1-yl)-1,3-benzothiazole (115 mg, 0.43 mmol) were added sequentially at ambient temperature to a stirred solution of 3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A) (100 mg, 0.36 mmol) in dry dimethylacetamide (1 ml), the mixture was stirred at ambient temperature for 1 hour and allowed to stand at ambient temperature for 16 hours, then it was added dropwise to stirred cold water (10 ml). After 30 minutes, the resulting solid was collected by filtration, washed with water (2 x 1 ml) and hexane (1 ml), dried in vacuo and purified using preparative HPLC to give the desired compound (5) (166 mg) as a grey solid; HPLC purity 90%; HPLC Retention time 4.21 minutes; m/z (M+H)⁺ 530.

b. The following compounds were synthesised in a manner analogous to that described in (a) above, but using appropriate alternative amine starting materials.

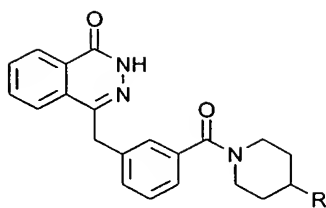


Compound	R	LC RT (minutes)	M+1	LC Purity (%)
6		4.18	508	90
7		3.22	551	90
8		4.13	508	90
9		3.95	483	90
10		3.79	465	90
11		3.76	406	90
12		2.80	407	90



Compound	R	LC RT (minutes)	M+1	LC Purity (%)
13 (Note 1)		3.56	494	100
14		3.71	451	90
15		4.39	538	90
16		3.66	498	90
17		4.33	533	90

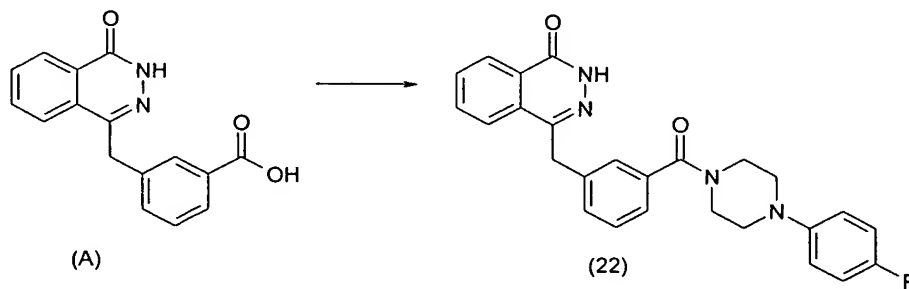
Note 1: 13 did not require purification via preparative scale HPLC - the product from the reaction was essentially pure.



Compound	R	LC RT (minutes)	M+1	LC Purity (%)
18		4.64	526	90
19		3.99	482	90
20		4.00	452	90
21		4.15	466	90

5 Example 3

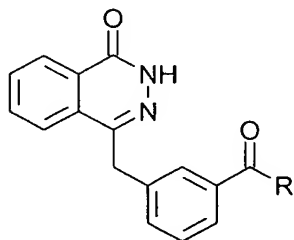
a. 4-{3-[4-(4-fluorophenyl)piperazin-1-ylcarbonyl]benzyl}-1(2H)-phthalazinone (22)



2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (150 mg, 0.47 mmol), diisopropylethylamine (102 mg, 0.8 mmol) and 1-(4-fluorophenyl)piperazine (65 mg, 0.47 mmol) were added sequentially at ambient temperature to a stirred solution of 3-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A) (100 mg, 0.36 mmol) in dry dimethylacetamide (1 ml), the mixture was stirred at ambient temperature for 4 hours and allowed to stand at ambient temperature for 16 hours, then it was added dropwise to stirred cold water (10 ml). After 30 minutes, the resulting solid was collected by filtration, washed with water (2 x 1 ml) and hexane (1 ml), dried in vacuo and purified using preparative HPLC to give 4-{3-[4-(4-fluorophenyl)piperazin-1-ylcarbonyl]benzyl}-1(2H)-phthalazinone (22) (76 mg) as a cream solid; m/z $(M+H)^+$ 443; HPLC Purity 90%; HPLC Retention time 4.00 minutes.

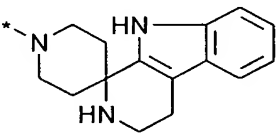
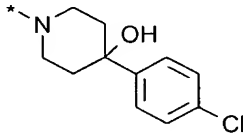
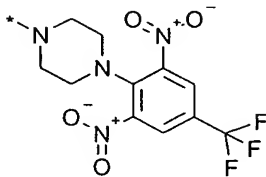
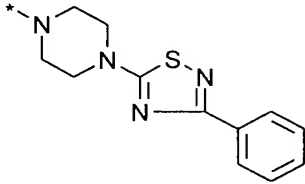
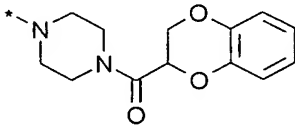
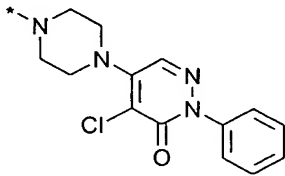
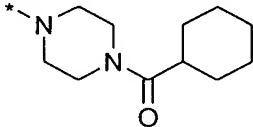
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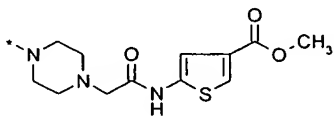
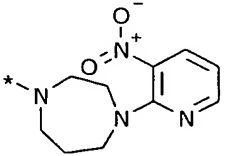
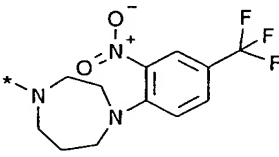
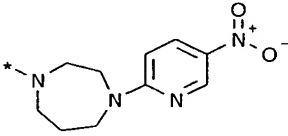
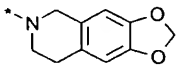
b. The following compounds were synthesised in a manner analogous to that described in (a) above, but using appropriate alternative amine starting materials.



20

Compound	R	LC RT (minutes)	M+1	LC Purity (%)
23		4.00	470	90
24		4.26	486	90

Compound	R	LC RT (minutes)	M+1	LC Purity (%)
25		3.18	504	85
26		3.78	473	90
27		4.46	583	90
28		4.96	509	90
29		3.73	511	90
30		3.78	553	90
31		3.71	459	90

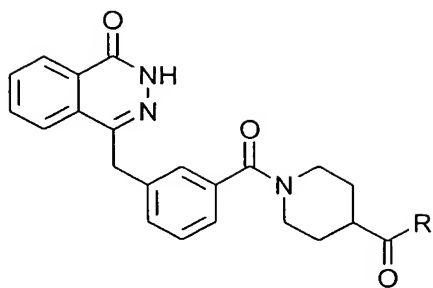
Compound	R	LC RT (minutes)	M+1	LC Purity (%)
32		3.94	546	90
33		3.84	485	90
34		4.37	552	90
35		3.77	485	90
36 (Note 2)		2.89	440	100

Note 2: 36 did not require purification via preparative scale HPLC - the product from the reaction was essentially pure.

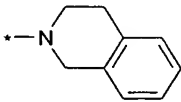
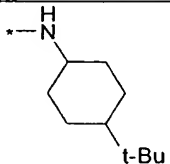
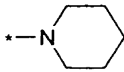
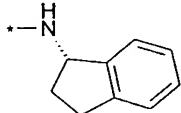
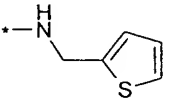
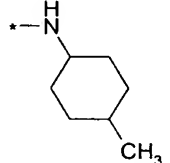
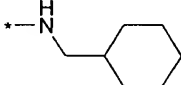
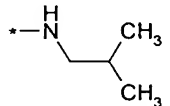
5 Example 4

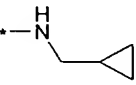
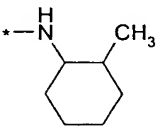
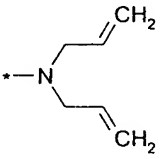
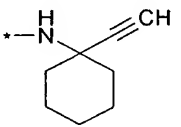
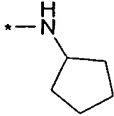
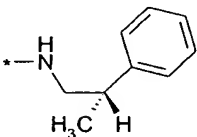
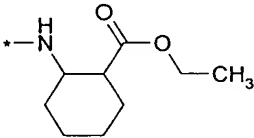
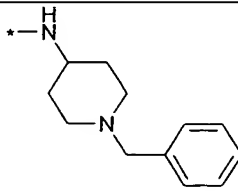
1-[3-(4-Oxo-3,4-dihydro-phthalazin-1-ylmethyl)-benzoyl]-piperidine-4-carboxylic acid (C) (0.24 mmol) was added to a solution of the appropriate amine (0.2 mmol) in dimethylacetamide (2 ml). 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.3 mmol) and Hunigs base (0.4 mmol) were then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

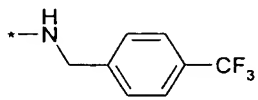
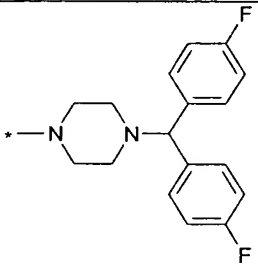
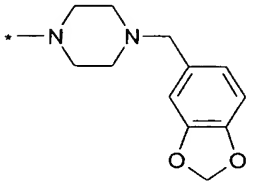
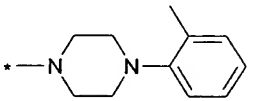
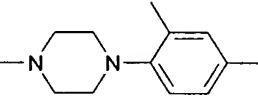
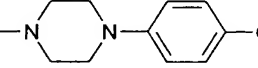
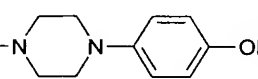
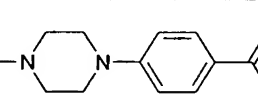
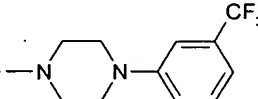
The compounds synthesised are set out below.

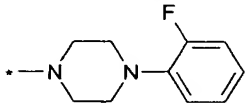
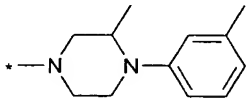
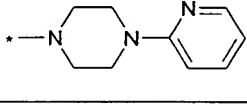
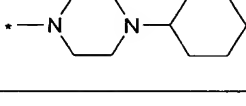


Compound	R	LC RT (minutes)	M+1	LC Purity (%)
37		4.39	572	90
38		3.71	496	90
39		3.63	474	80
40		3.76	474	90
41		3.56	502	90
42		3.58	568	90

Compound	R	LC RT (minutes)	M+1	LC Purity (%)
43		3.81	508	90
44		4.39	531	90
45		3.52	460	85
46		3.77	508	90
47		3.59	488	90
48		3.83	488	90
49		3.85	488	90
50		3.47	448	90

Compound	R	LC RT (minutes)	M+1	LC Purity (%)
51		3.36	446	90
52		3.77	488	90
53		3.74	472	90
54		3.82	498	90
55		3.52	460	90
56		3.86	510	90
57		3.75	546	90
58		3.01	565	90

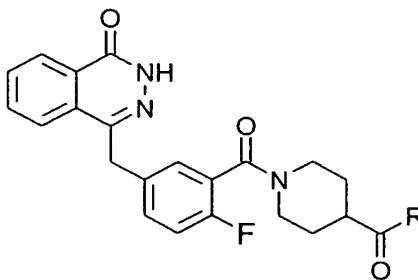
Compound	R	LC RT (minutes)	M+1	LC Purity (%)
59		3.93	549	90
60		4.17	663	90
61		3.08	595	90
62		4.16	551	90
63		4.3	565	90
64		4.1	571	90
65		3.64	567	90
66		3.62	579	90
67		4.27	605	90

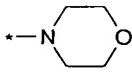
Compound	R	LC RT (minutes)	M+1	LC Purity (%)
68		3.89	555	90
69		3.84	565	90
70		2.92	565	90
71		3.02	543	90

Example 5

1-[2-Fluoro-5-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoyl]-
 5 piperidine-4-carboxylic acid (D) (0.24 mmol) was added to a solution
 of the appropriate amine (0.2 mmol) in dimethylacetamide (2 ml). 2-
 (1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
 (0.3 mmol) and Hunigs base (0.4 mmol) were then added and the reaction
 was stirred at room temperature for 16 hours. The reaction mixtures
 10 were then purified by preparative HPLC.

The compounds synthesised are set out below.

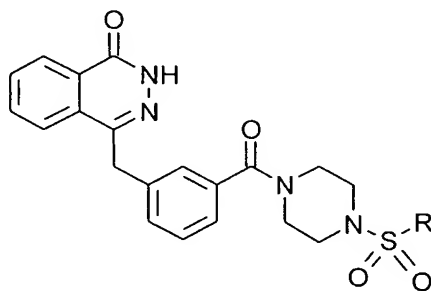


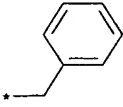

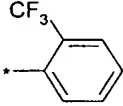
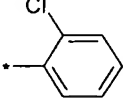
Compound	R	LC RT (minutes)	M+1	LC Purity (%)
72		3.21	480	90

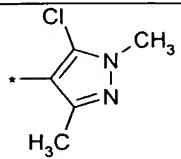
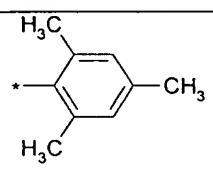
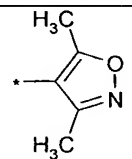
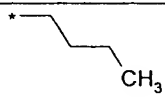
Example 6

5 The appropriate sulphonyl chloride (0.24 mmol) was added to a solution of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

10 The compounds synthesised are set out below.



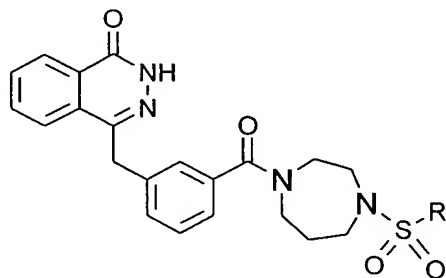
Compound	R	LC RT (minutes)	M+1	LC Purity (%)
73		3.85	504	90
74		3.44	442	90
75		4.09	558	90
76		3.93	525	90

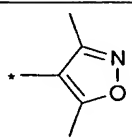
77		3.73	543	90
78		4.38	532	90
79		3.76	509	90
80		3.82	470	90

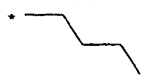
Example 7

The appropriate sulphonyl chloride (0.24 mmol) was added to a solution of 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

10 The compounds synthesised are set out below.



Compound	R	LC RT (minutes)	M+1	LC Purity (%)
81		3.71	523	90

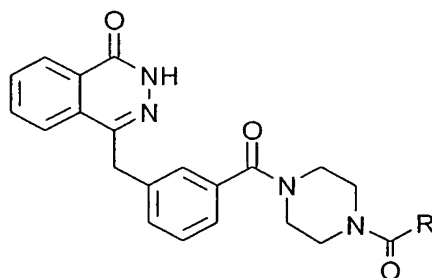
82		3.68	484	90
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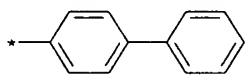
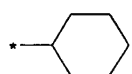
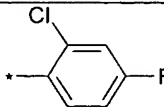
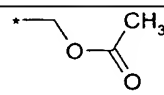
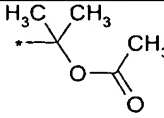
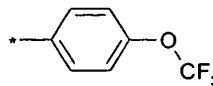
Example 8

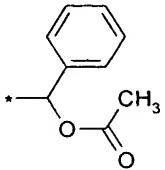
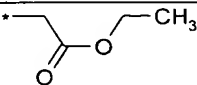
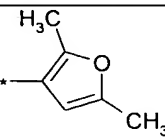
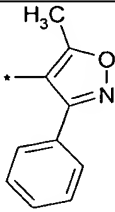
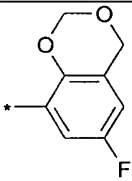
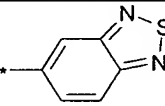
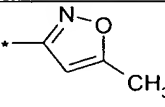
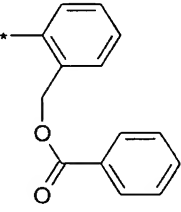

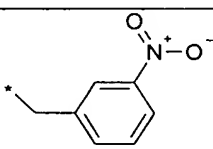
The appropriate acid chloride (0.24 mmol) was added to a solution of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

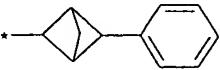
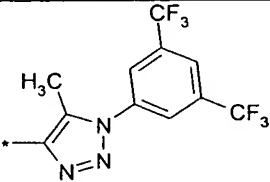
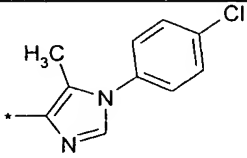
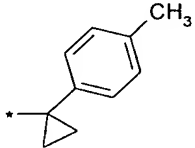
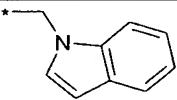
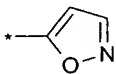
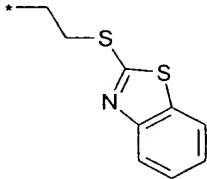
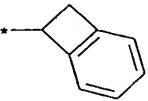
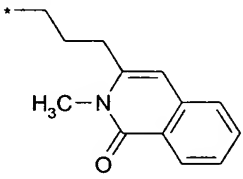
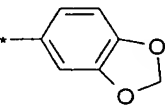
The compounds synthesised are set out below.

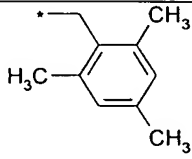
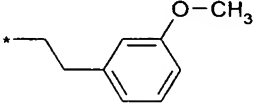
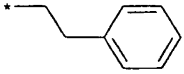
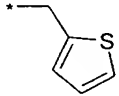
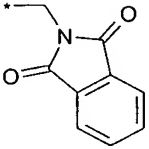
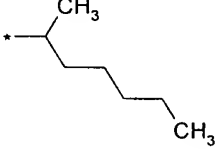
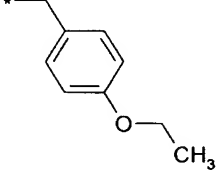
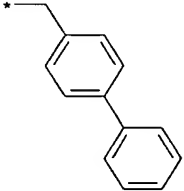
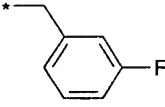
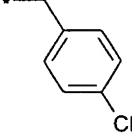
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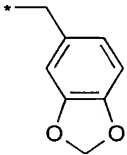
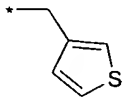
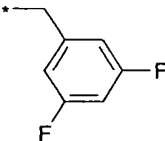
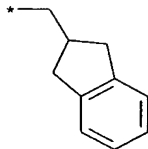
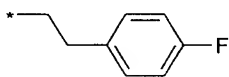
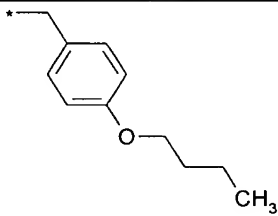
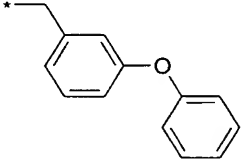
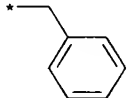
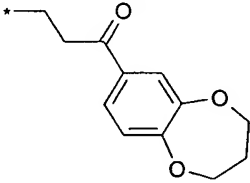


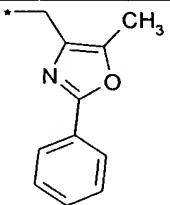
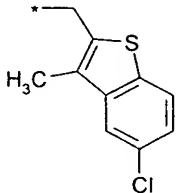
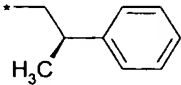
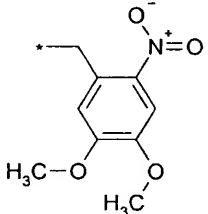
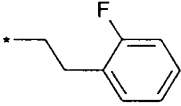
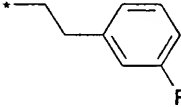
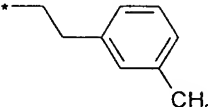
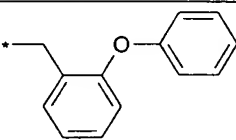
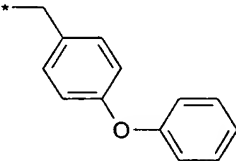
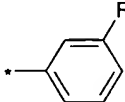
Compound	R	LC RT (minutes)	M+1	LC Purity (%)
83		4.04	530	85
84		3.71	460	90
85		3.69	506	90
86		3.19	450	85
87		3.44	478	90
88		3.94	538	90

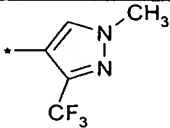
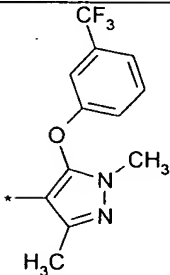
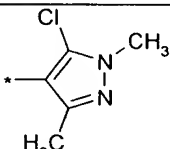
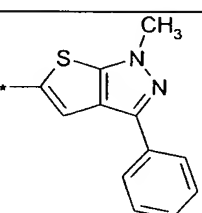
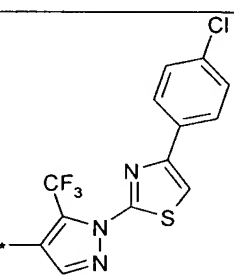
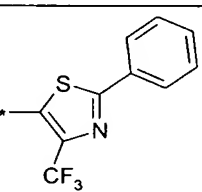
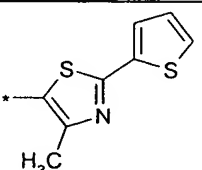
89		3.68	526	90
90		3.6	464	90
91		3.63	472	90
92		3.73	535	80
93		3.49	530	90
94		3.5	512	85
95		3.52	459	90
96		4.01	588	90
97		4.05	406	90
98		3.84	513	90

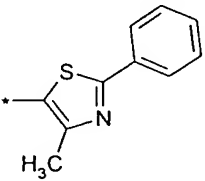
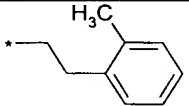
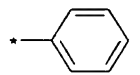
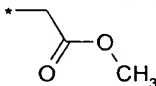
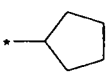
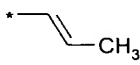
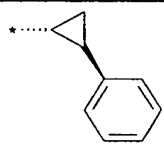

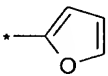
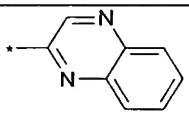
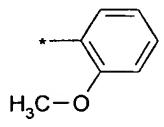
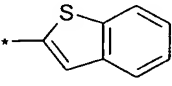
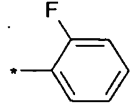
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100		4.41	671	90
101		3.62	582	90
102		3.82	508	90
103		3.81	507	90
104		3.33	445	90
105		4.08	571	90
106		3.67	480	90
107		3.54	577	90
108		3.49	498	90

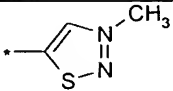
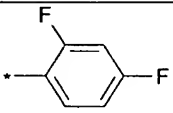
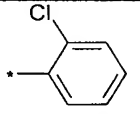
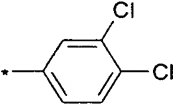
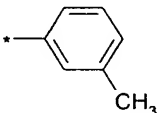

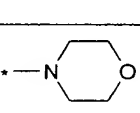
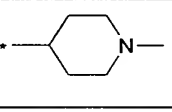
109		4.04	510	90
110		3.75	512	90
111		3.67	482	90
112		3.54	474	90
113		3.52	537	90
114		4.13	475	90
115		3.8	512	85
116		4.09	544	90
117		3.63	486	90
118		3.91	502	90

119		3.61	511	90
120		3.57	474	90
121		3.67	504	90
122		4.02	508	90
123		3.81	500	90
124		4.11	540	90
125		4.19	560	90
126		3.61	468	90
127		3.69	582	90

128		3.85	549	85
129		4.37	573	90
130		3.84	496	90
131		3.62	573	90
132		3.72	500	90
133		3.8	500	85
134		3.9	496	90
135		4.03	560	90
136		4.16	560	90
137		4.71	472	80

138		3.47	526	90
139		3.78	632	90
140		3.27	506	90
141		3.92	590	90
142		4.76	706	90
143		4.27	605	90
144		3.71	557	90

145		3.98	551	90
146		3.9	496	90
147		3.57	454	90
148		3.21	450	90
149		3.61	446	90
150		3.39	418	85
151		3.81	494	90
152		3.31	418	90
153		3.38	444	90
154		3.56	506	85
155		3.48	484	90
156		3.84	510	90
157		3.56	472	90

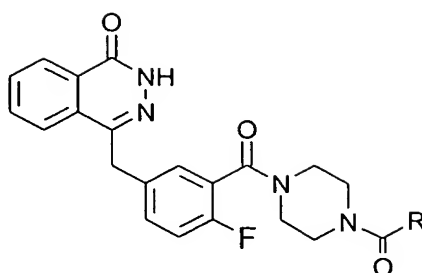
158		3.34	476	90
159		3.56	490	90
160		3.53	488	90
161		3.99	523	90
162		3.7	468	90
163		3.11	392	90
164		3.16	463	90
165		2.78	475	90

Example 9

- 5 The appropriate acid chloride (0.24 mmol) was added to a solution of 4-[4-Fluoro-3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (2) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

10

The compounds synthesised are set out below.

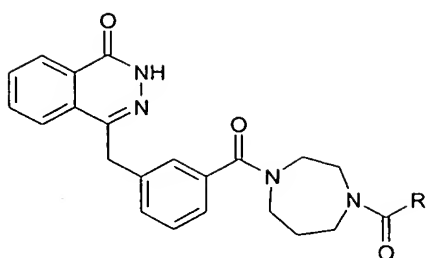


Compound	R	LC RT (minutes)	M+1	LC Purity (%)
166		4.04	424	90
167		2.84	493	90
168		4.14	436	90
169		3.19	481	90
170		2.83	509	90
171		2.63	522	90
172		2.71	522	90
173		2.8	479	90

5 Example 10

The appropriate acid chloride (0.24 mmol) was added to a solution of 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.

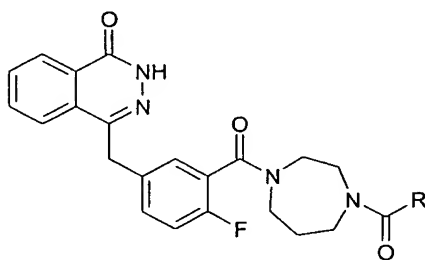


Compound	R	LC RT (minutes)	M+1	LC Purity (%)
174		3.16	416	90
175		3.84	534	90
176		3.63	496	90
177		3.52	460	90
178		3.62	514	90
179		3.23	459	90
180		3.95	490	90
181		3.63	533	90
182		3.61	526	90

Example 11

The appropriate acid chloride (0.24 mmol) was added to a solution of 4-[3-([1,4]diazepane-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one (4) (0.2 mmol) in dichloromethane (2 ml). Hunigs base (0.4 mmol) was then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.

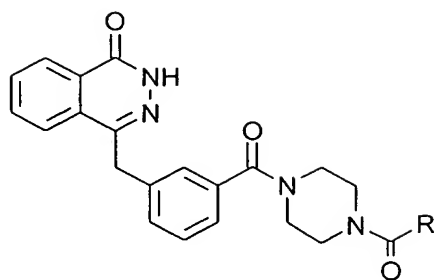


Compound	R	LC RT (minutes)	M+1	LC Purity (%)
183		2.81	467	90
184		3.19	495	90

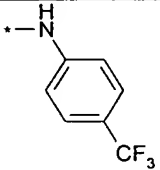
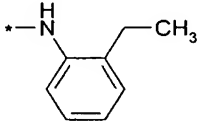
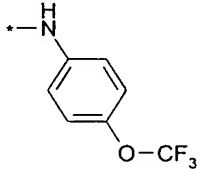
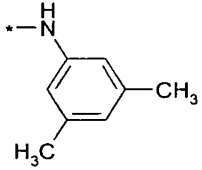
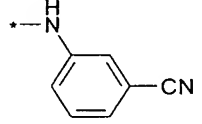
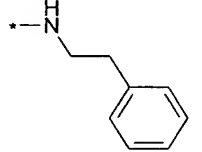
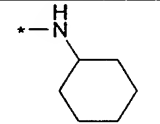
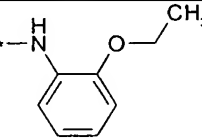
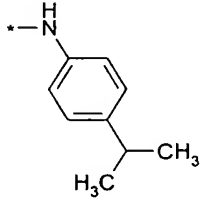
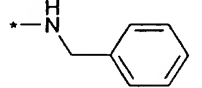
Example 12

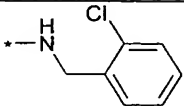
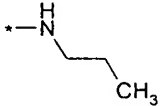
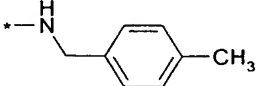
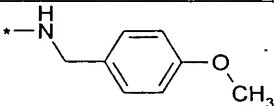
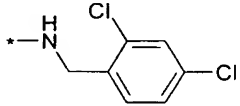
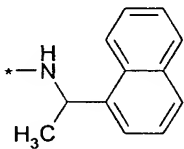
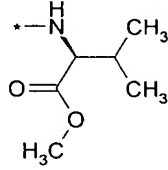
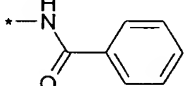
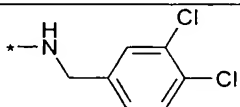
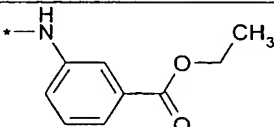
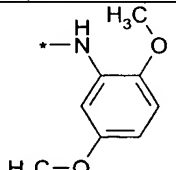
The appropriate isocyanate (0.24 mmol) was added to a solution of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) (0.2 mmol) in dichloromethane (2 ml). The reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

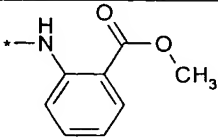
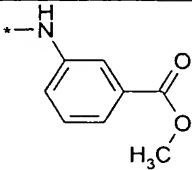
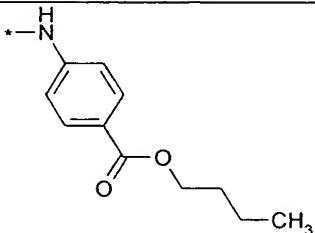
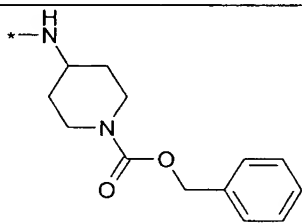
The compounds synthesised are set out below.



Compound	R	LC RT (minutes)	M+1	LC Purity (%)
185		3.34	435	85
186		3.76	483	90
187		3.49	449	90
188		3.57	487	90
189		4.03	537	90
190		3.77	519	85
191		3.72	487	90
192		3.57	505	90

193		4.08	537	80
194		3.68	497	85
195		4.03	553	90
196		3.93	497	90
197		3.62	494	90
198		3.68	497	90
199		3.73	475	90
200		3.9	513	90
201		4.11	511	90
202		3.58	483	90

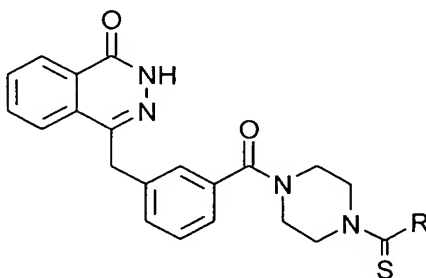
203		3.71	517	90
204		3.34	435	85
205		3.71	497	90
206		3.56	513	90
207		4.04	552	90
208		4	547	90
209		3.54	507	90
210		3.42	497	90
211		3.95	552	90
212		3.79	541	85
213		3.66	529	90

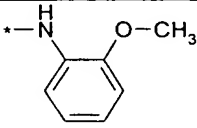
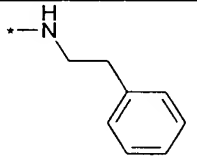
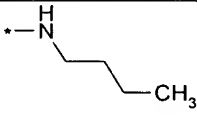
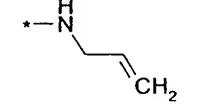
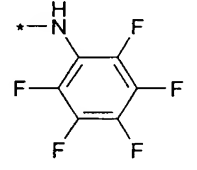
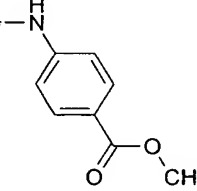
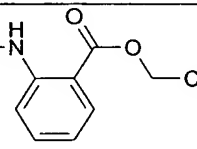
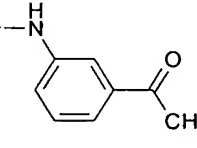
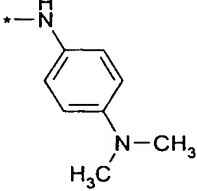
214		3.92	527	85
215		3.62	527	90
216		4.28	569	90
217		3.81	610	90


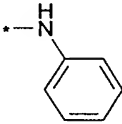
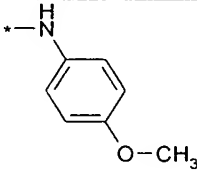
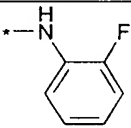
Example 13

The appropriate isothiocyanate (0.24 mmol) was added to a solution of 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) (0.2 mmol) in dichloromethane (2 ml). The reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

10 The compounds synthesised are set out below.



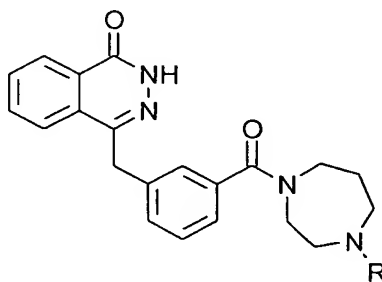
Compound	R	LC RT (minutes)	M+1	LC Purity (%)
218		3.68	515	90
219		4.05	513	90
220		3.94	465	90
221		3.55	449	90
222		4.21	575	90
223		3.79	543	90
224		4.28	557	85
225		3.63	527	90
226		3.18	528	90

227		3.32	423	90
228		3.69	485	80
229		3.68	515	90
230		3.72	503	90

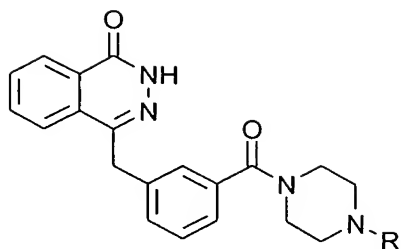
Example 14

3-(4-Oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (A) (0.24 mmol) was added to a solution of the appropriate amine (0.2 mmol) in dimethylacetamide (2 ml). 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.3 mmol) and Hunigs base (0.4 mmol) were then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparatory HPLC.


The compounds synthesised are set out below.

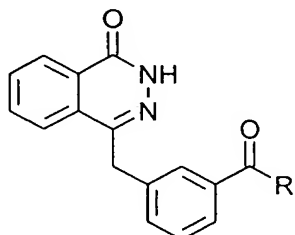


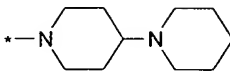
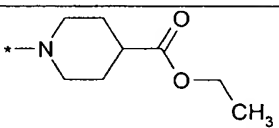
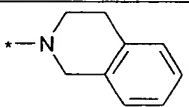
Compound	R	LC RT (minutes)	M+1	LC Purity (%)
231	---CH_3	2.72	378	90



Compound	R	LC RT (minutes)	M+1	LC Purity (%)
232		2.96	427	90
233		4.08	444	90
234		3.9	456	95
235		3.83	450	95
236		2.98	432	90
237		4.17	440	90
238		2.9	449	90
239		4.31	460	90
240		3.63	468	90
241		3.78	456	90

242		3.08	378	90
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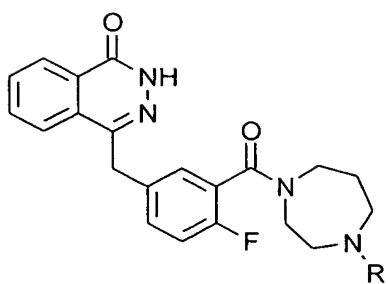


Compound	R	LC RT (minutes)	M+1	LC Purity (%)
243		2.88	432	90
244		3.61	421	95
245		3.96	397	90

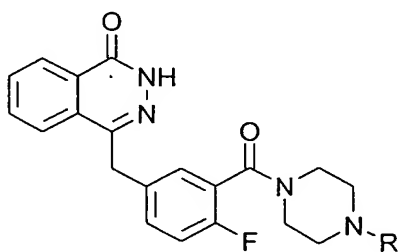
5 Example 15

2-Fluoro-5-(4-oxo-3,4-dihydrophthalazin-1-ylmethyl)benzoic acid (B) (0.24 mmol) was added to a solution of the appropriate amine (0.2 mmol) in dimethylacetamide (2 ml). 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.3 mmol) and Hunigs base (0.4 mmol) were then added and the reaction was stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.

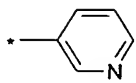
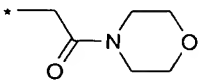
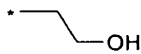
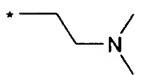


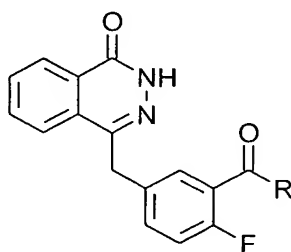
Compound	R	LC RT (minutes)	M+1	LC Purity (%)
246	*-CH ₃	2.8	396	90
247	*-CH ₂ CH ₂ OH	2.79	426	90

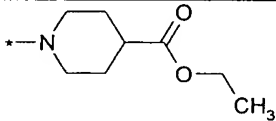
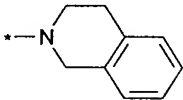


5

Compound	R	LC RT (minutes)	M+1	LC Purity (%)
248	*-C ₆ H ₄ -OCH ₃	3.9	456	95
249	*-C(=O)OC(CH ₃) ₃	3.97	467	90
250	*-CH ₂ CH ₂ OCH ₃	2.84	426	90
251	*-CH ₃	3.46	368	90

252		2.91	445	90
253		2.77	495	90
254		2.69	412	90
255		2.76	439	90



Compound	R	LC RT (minutes)	M+1	LC Purity (%)
256		3.81	439	90
257		3.95	415	90

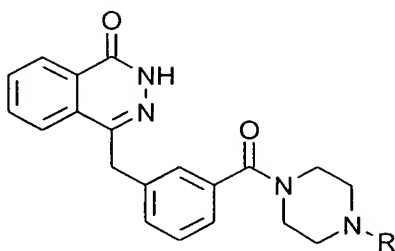
5

Example 16

An appropriate aldehyde (0.2 mmol) and 4-[3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (1) (0.24 mmol) were dissolved in dichloromethane (2 ml). Sodium triacetoxymethylborohydride (0.28 mmol) and glacial acetic acid (6.0 mmol) were then added and stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.

15

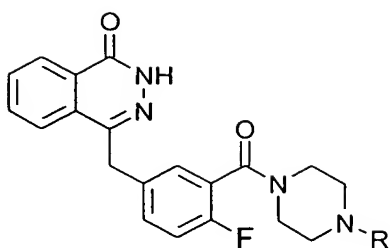


Compound	R	LC RT (minutes)	M+1	LC Purity (%)
258	 H ₃ C-CH ₂ -CH ₂ -CH ₃	2.89	406	90
259	 CH ₃ -CH ₂ -CH ₂ -CH ₂ -	2.91	406	90
260	 CH ₃ -CH ₂ -CH ₃	2.75	392	90
261	 CH ₃	3.16	493	90
262	 H	3.09	479	90

Example 17

5 An appropriate aldehyde (0.2 mmol) and 4-[4-Fluoro-3-(piperazine-1-carbonyl)benzyl]-2H-phthalazin-1-one (2) (0.24 mmol) were dissolved in dichloromethane (2 ml). Sodium triacetoxyborohydride (0.28 mmol) and glacial acetic acid (6.0 mmol) were then added and stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.

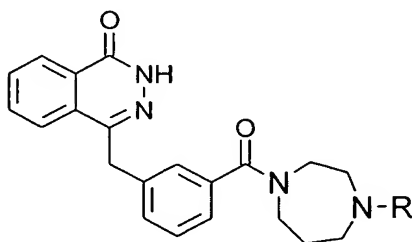


Compound	R	LC RT (minutes)	M+1	LC Purity (%)
263		2.83	459	90
264		3.04	465	90

Example 18

5 An appropriate aldehyde (0.2 mmol) and 4-[3-([1,4]diazepane-1-carbonyl)benzyl]-2H-phthalazin-1-one (3) (0.24 mmol) were dissolved in dichloromethane (2 ml). Sodium triacetoxyborohydride (0.28 mmol) and glacial acetic acid (6.0 mmol) were then added and stirred at room temperature for 16 hours. The reaction mixtures were then purified by
10 preparative HPLC.

The compounds synthesised are set out below.

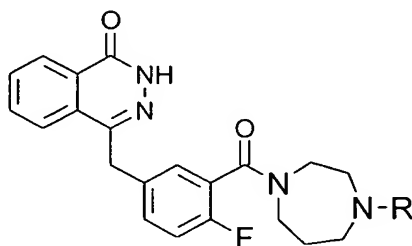


Compound	R	LC RT (minutes)	M+1	LC Purity (%)
265		2.76	406	90

Example 19

An appropriate aldehyde (0.2 mmol) and 4-[3-([1,4]diazepane-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one (4) (0.24 mmol) were dissolved in dichloromethane (2 ml). Sodium triacetoxyborohydride (0.28 mmol) and glacial acetic acid (6.0 mmol) were then added and stirred at room temperature for 16 hours. The reaction mixtures were then purified by preparative HPLC.

The compounds synthesised are set out below.



Compound	R	LC RT (minutes)	M+1	LC Purity (%)
266		2.8	424	90

Example 20

In order to assess the inhibitory action of the compounds, the following assay was used to determine IC₅₀ values.

Mammalian PARP, isolated from Hela cell nuclear extract, was incubated with Z-buffer (25mM Hepes (Sigma); 12.5 mM MgCl₂ (Sigma); 50mM KCl (Sigma); 1 mM DTT (Sigma); 10% Glycerol (Sigma) 0.001% NP-40 (Sigma); pH 7.4) in 96 well FlashPlates (TRADE MARK) (NEN, UK) and varying concentrations of said inhibitors added. All compounds were diluted in DMSO and gave final assay concentrations of between 10 and 0.01 µM, with the DMSO being at a final concentration of 1% per well. The total assay volume per well was 40 µl.

After 10 minutes incubation at 30°C the reactions were initiated by the addition of a 10 µl reaction mixture, containing NAD (5µM), ³H-NAD and

30mer double stranded DNA-oligos. Designated positive and negative reaction wells were done in combination with compound wells (unknowns) in order to calculate % enzyme activities. The plates were then shaken for 2 minutes and incubated at 30°C for 45 minutes.

5

Following the incubation, the reactions were quenched by the addition of 50 µl 30% acetic acid to each well. The plates were then shaken for 1 hour at room temperature.

10 The plates were transferred to a TopCount NXT (TRADE MARK) (Packard, UK) for scintillation counting. Values recorded are counts per minute (cpm) following a 30 second counting of each well.

The % enzyme activity for each compound is then calculated using the
15 following equation:

$$\% \text{ Inhibition} = 100 - \left(100 \times \frac{(\text{cpm of unknowns} - \text{mean negative cpm})}{(\text{mean positive cpm} - \text{mean neagative cpm})} \right)$$

IC₅₀ values (the concentration at which 50% of the enzyme activity is
20 inhibited) were calculated, which are determined over a range of different concentrations, normally from 10 µM down to 0.001 µM. Such IC₅₀ values are used as comparative values to identify increased compound potencies.

25 All compounds tested had a IC₅₀ of less than 0.1 µM.

The following compounds have an IC₅₀ of less than 0.01µM: 2, 3, 4, 5 9,
10, 13, 14, 15, 16, 17, 18, 19, 20, 21, 25, 27, 28, 29, 31, 33, 34,
35, 37, 40, 41, 44, 46, 47, 48, 49, 51, 53, 73, 74, 75, 76, 77, 78,
30 79, 80, 83, 84, 85, 97, 98, 99, 101, 103, 105, 106, 107, 111, 112,
113, 114, 117, 118, 119, 120, 121, 122, 130, 131, 132, 133, 138, 139,
140, 143, 149, 153, 155, 156, 157, 159, 160, 161, 175, 176, 177, 178,
179, 181, 182, 186, 188, 190, 191, 195, 197, 198, 200, 201, 202, 203,
205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 216, 217, 218, 219,
35 221, 222, 223, 225, 226, 227, 228, 229, 232, 237, 240, 243, 245, 246,
256.

The following compounds, as well as those above, have an IC_{50} of less than $0.02\mu M$: 1, 6, 7, 8, 11, 12, 22, 23, 24, 26, 32, 36, 38, 39, 42, 43, 45, 50, 54, 55, 56, 58, 59, 81, 82, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 100, 104, 108, 109, 110, 115, 116, 123, 126, 127, 128, 134, 135, 137, 141, 145, 146, 147, 150, 151, 152, 154, 158, 162, 163, 174, 180, 185, 187, 189, 192, 193, 194, 196, 199, 204, 215, 220, 224, 230, 233, 234, 235, 239, 241, 242, 244, 250, 251, 258, 259 and 261.

The Potentiation Factor (PF_{50}) for compounds is calculated as a ratio of the IC_{50} of control cell growth divided by the IC_{50} of cell growth + PARP inhibitor. Growth inhibition curves for both control and compound treated cells are in the presence of the alkylating agent methyl methanesulfonate (MMS). The test compounds were used at a fixed concentration of 0.2 micromolar. The concentrations of MMS were over a range from 0 to 10 $\mu g/ml$.

Cell growth was assessed using the sulforhodamine B (SRB) assay (Skehan, P., et al., (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. **82**, 1107-1112.). 2,000 HeLa cells were seeded into each well of a flat-bottomed 96-well microtiter plate in a volume of 100 μl and incubated for 6 hours at $37^{\circ}C$. Cells were either replaced with media alone or with media containing PARP inhibitor at a final concentration of 0.5, 1 or 5 μM . Cells were allowed to grow for a further 1 hour before the addition of MMS at a range of concentrations (typically 0, 1, 2, 3, 5, 7 and 10 $\mu g/ml$) to either untreated cells or PARP inhibitor treated cells. Cells treated with PARP inhibitor alone were used to assess the growth inhibition by the PARP inhibitor.

Cells were left for a further 16 hours before replacing the media and allowing the cells to grow for a further 72 hours at $37^{\circ}C$. The media was then removed and the cells fixed with 100 μl of ice cold 10% (w/v) trichloroacetic acid. The plates were incubated at $4^{\circ}C$ for 20 minutes and then washed four times with water. Each well of cells was then stained with 100 μl of 0.4% (w/v) SRB in 1% acetic acid for 20 minutes before washing four times with 1% acetic acid. Plates were then dried for 2 hours at room temperature. The dye from the stained cells was solubilized by the addition of 100 μl of 10mM Tris Base into

each well. Plates were gently shaken and left at room temperature for 30 minutes before measuring the optical density at 564nm on a Microquant microtiter plate reader.

- 5 All the compounds tested had a PF_{50} at 200nM of at least 2.0.

Example 21

Materials and Methods

Small molecule inhibitors of PARP:

- 10 Compound (4) was dissolved in DMSO at 10mM and stored at -20°C in the dark.

Cell Lines

- VC8 cells and the mouse Brca2 BAC complemented derivatives were as described in M. Kraakman-van der Zwet, et al., *Mol Cell Biol* **22**, 669-79 (2002)). ES cells defective in Brca2 function have been described previously (Tutt, et al., *EMBO Rep* **3**, 255-60 (2002)). The construction of ES cells defective in Brca1 will be described elsewhere but have previously been validated (Foray, et al., *Embo J*, **22**, 2860-71 (2003)).

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Clonogenic Assays

- For measurement of cellular sensitivity to a PARP inhibitor (compound 4), cell cultures in exponential growth were trypsinised and seeded at various densities in 6-well plates onto Mitomycin C inactivated mouse embryonic fibroblasts and where appropriate treated with the test compound after 18 hours. For continuous exposure, cells were re-fed every 4 days with fresh medium and inhibitor. After 10-14 days, cells were washed with PBS, fixed in methanol and stained with crystal violet. Colonies containing greater than approximately 50 cells were counted. Experiments were performed at least three times in triplicate.

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Results

Reduction in the viability of BRCA1 and BRCA2 deficient cells

- 35 Compound 4 was used to probe the sensitivity of cells deficient in Brca1 or Brca2 to the inhibition of PARP activity. Clonogenic assays showed that both Brca1 and Brca2 deficient cell lines were extremely sensitive to compound 4 compared to otherwise isogenic cells (Fig. 1A,

1B). The SF_{50} (dosage at which 50% of cells survived) for Compound 4 was $1.5 \times 10^{-8}M$ for cells deficient in Brca1, whilst the SF_{50} for matched wild type cells was $7 \times 10^{-6}M$ (Figure 1A). This represents a factor of 467 fold enhanced sensitivity of Brca1 mutant cells compared to wild type cells.

The SF_{50} for Compound 4 was $1.2 \times 10^{-8}M$ for cells deficient in Brca2 whilst the SF_{50} for matched wild type cells was $1.8 \times 10^{-5}M$ (Figure 1B).

This represents a factor of 1,500 fold enhanced sensitivity of Brca2 mutant cells compared to wild type cells. Similar results were obtained with Chinese hamster ovary cells deficient in Brca2 (VC8) compared to a Brca2-complemented derivative (VC8-BAC) (Figure 2). The SF_{50} for Compound 4 was $5 \times 10^{-8}M$ for the Brca2 deficient VC8 line whilst the SF_{50} for matched control, VC8-BAC, was $3 \times 10^{-5}M$ (Figure 2). This represents a factor of 600 fold enhanced sensitivity of Brca2 mutant cells compared to wild type cells.